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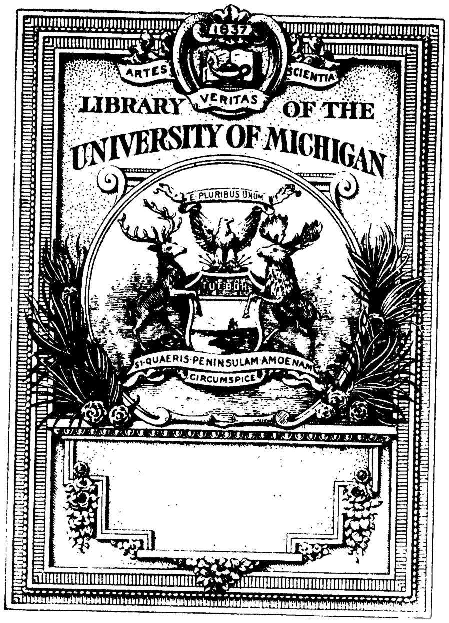

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THE PHILIPPINE

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B. MEDICAL SCIENCES

VOL. II MARCH, 1907 No. 1

OBSERVATIONS UPON FILARIA PHILIPPINENSIS AND ITS DEVELOPMENT IN THE MOSQUITO.

By P. M. ASHBURN and CHARLES F. CRAIG.[1]

(*From the laboratory of the United States Army Board for the Study of Tropical Diseases, Division Hospital, Manila, P. I., and the Biological Laboratory of the Bureau of Science.*)

INTRODUCTION.

In a special report submitted to the Surgeon-General of the Army in May, 1906, and published in the American Journal of the Medical Sciences in September of that year, we described a new blood filaria of man, to which we gave the name *Filaria philippinensis* sp. nov. because of its occurrence in a native of the Philippine Islands.

We considered that this filaria represented a new species, and that it was probably the only one indigenous to these Islands; a new species, because of its morphology, lack of periodicity and the rarity of filariasis in the Philippines; the only indigenous one, because the previous descriptions of the filariæ observed in natives of these Islands contained nothing which would exclude the possibility that the observers might have been dealing with *Filaria philippinensis*.

When our preliminary communication regarding this parasite was written we had seen but one case of infection with it, but since then we have had the opportunity of studying four additional cases, all in natives of the Philippine Islands. To Doctor Albert L. Miller, contract surgeon,

---

[1] P. M. Ashburn, captain and assistant surgeon, United States Army, and Charles F. Craig, first lieutenant and assistant surgeon, United States Army, constituting the United States Army Board for the Study of Tropical Diseases as They Occur in the Philippine Islands.

United States Army, we are indebted for the additional cases which he found while examining the blood of a company of Philippine Scouts at Imus, Cavite Province, with the view of determining how many of this company were infected with *Filaria philippinensis.* These men, who were transferred to us for observation, belonged to one company of Scouts and all were born upon the Island of Luzon, in the Province of Ambos Camarines.

From a study of these cases we have been able to confirm our original description of this filaria and to make additional observations upon its morphology and life history, especially as regards its developmental changes within a mosquito, *Culex fatigans* Wied.

RÉSUMÉ OF THE ORIGINAL DESCRIPTION.

In order that the observations which follow may clearly be understood the following brief résumé of our original description is necessary:

Filaria philippinensis is a small blood filaria found in natives of the Philippine Islands; it presents no periodicity, occurring in practically equal, but small numbers, at all hours of the day and night; it is actively motile, possessing both lashing and progressive motion and presents for description a sheath, an anterior extremity, a body and a posterior extremity. The average length of the living filaria is 0.32 millimeter, but variations occur between 0.29 and 0.335 millimeter.

The sheath is visible at either extremity, being invisible along the rest of the body; it has the appearance of a fine thread, as thin as the flagellum of a trypanosoma; along the body the sheath is transparent and very tightly fitting, thus preventing the filaria from slipping backward and forward within it, as in the case of *Filaria nocturna* Manson and *Filaria diurna* Manson.

The anterior extremity or head consists of a hemispherical base, in the center of which is placed a small spicule which retracts within this base; surrounding this portion of the head is a serrated prepuce which, when closed, covers it, when retracted, exposes it; the number of serrations or lips can not be determined. The prepuce is constantly drawn back and forth, as is likewise the spicule, but the motion of the two is not always synchronous.

The body is graceful and sinuous, consisting of an outer, radially striated, musculo-cutaneous coat and an inner portion or body cavity; the latter is clear and refractile and contains the following constant viscera:

(*a*) *Anterior V spot.*—A bright, refractile, triangular area, placed at one side of the worm, about 0.105 millimeter from the anterior margin of the head, and opening by its apex upon the surface, piercing the musculo-cutaneous coat.

(*b*) *Central viscus.*—In the posterior portion of the central third of the body is situated a convoluted or spiral tube or cylinder, resembling a vine tendril, and presenting five or six spiral turns which progressively grow smaller, ending in a fine, curved or straight extremity. This central viscus is placed in the same portion of the worm as is the "granular mass" in *Filaria nocturna.*

(*c*) *Posterior V spot and papilla.*—At the center of the posterior third of the body there is situated a triangular spot, its apex opening upon the surface, piercing the musculo-cutaneous coat, where it marks the site of a distinct papilla which bulges beyond the body line and may represent the anus or cloaca of the adult worm.

The posterior extremity or tail, at a point midway between the posterior V spot and the tip becomes suddenly attenuated to three quarters of its former diameter, thus forming a distinct offset upon each side; from this point the tail diminishes progressively and uniformly to a very fine, thread-like point. Because of its situation in relation to the embryonic anus and its apparently less vital characteristics, the portion of the tail beyond the attenuation probably dies or is sloughed off during the later development of the filaria.

In stained specimens, a column of deeply stained nuclei is observed running the entire length of the worm, broken here and there by unstained areas, which vary in position in individual specimens; the only "gap" in the stained column that is at all constant is situated at a point 20 per cent of the total length of the worm from the head; however, many specimens do not show this "gap," and there is also much variation in its situation in individual filaria. We do not regard it as of any diagnostic importance. In the stained specimen the anterior V spot, the central viscus, and the posterior V spot are not seen.

Filaria philippinensis is very actively motile, the movements being of a wriggling, lashing character and not progressive, as a rule, but at times a marked progressive motion is observed. (See figs. 1 and 18.)

LATER OBSERVATIONS.

The study of four more cases of infection with *Filaria philippinensis* has enabled us to add somewhat to the above description as regards the morphology, motility, and periodicity of this filaria.

Morphology.—We can add but little to our original description as regards the morphology of *Filaria philippinensis.* We have examined a very large number of filariæ in the blood with special reference to the armature of the head, but we have been unable to distinguish any definite number of lips; the spicule, mounted upon a smooth, hemispherical base, into which it retracts, is easily distinguished, but the retractile prepuce which incloses it can not be separated into definite lips, its margin being so very finely serrated. The serrations can easily be seen in the fresh specimen, but their number can not be determined, while in the stained specimen they are not visible.

In a certain proportion of stained filariæ in blood obtained from the mosquito's stomach shortly after the insect has bitten, and in which the sheath has been lost, what appears to be two lips can be seen, an upper one, broad and fleshy, and a lower, more slender and delicate. However, we are not convinced that these represent true lips, for the appearance may be due to the staining method, but their presence in a comparatively large number of the filariæ is suggestive.

The sheath, as we have already stated, is tight and generally only seen as a thread-like flagellum at the extremities of the worm. When the filaria is moving forward, the anterior end of the sheath bends backward along the side of the body, while the posterior end is lashed about by the movement of the tail; when the movements of the worm are lashing and not progressive, both ends of the sheath are whipped freely about. The filaria is never seen to slide forward and backward within the sheath, this being impossible because of the closeness with which it envelops the body. In very rare instances in the fresh specimen, the sheath may be seen to be flattened out, having a ribbon-like appearance, but even when this occurs the filaria does not enter the flattened portion projecting at either extremity.

We are not able to add anything to our previous description of the anterior V spot, the central viscus, the posterior V spot or the tail of the worm as observed in the blood of man; the same is true of the measurements of the filaria as given by us and the morphology of the stained specimen.

Motility.—In our original description we stated that *Filaria philippinensis* possessed two forms of motility, one lashing but not progressive, which is most frequently observed; the other, a marked progressive motion occurring but rarely. In our examinations of the filariæ in our additional cases we have given special attention to the character of the movements exhibited by them and have found that progressive motion is much more common in fresh blood specimens than we at first supposed to be the case. We have found that in thick blood smears progressive motion occurs very frequently, if not as a rule, and we have also observed it in very thin smears; when it takes place the movement is serpentine in character, the head advancing through the surrounding masses of blood corpuscles with considerable rapidity. In a fairly thick smear, in one instance, a filaria in three minutes crossed four 18-millimeter ($\frac{2}{3}$-inch) microscopic fields in almost a straight line; in another case, eight 18-millimeter ($\frac{2}{3}$-inch) fields were traversed in half a minute; in a blood smear in which the cells were arranged in a single layer a filaria crossed a 18-millimeter ($\frac{2}{3}$-inch) field in one minute and six 4.25-millimeter ($\frac{1}{6}$-inch) fields in three minutes. Even when a portion of the specimen was reached in which the blood cells were in very small numbers, or indeed absent, the filaria did not show entire loss of progressive motion, although such clear spaces caused a temporary cessation; in such instances, after a few moments of twisting and turning, the head of the parasite would shoot forward and the progressive motion be resumed; in this way we have seen a filaria traverse four such open spaces in a specimen of blood and in each instance as soon as it reached the intervening clumps of blood corpuscles the progressive motion became more rapid and regular in character. At times the tail end advanced but this was invariably followed almost immediately by a propulsive effort of the tail which pushed the head and body forward for a considerable distance, after which the head continued its advance. In one specimen we observed progressive motion of filariæ for two hours after the blood was collected.

It should be remembered that the movements, both lashing and progressive, which have been described occur while the filaria is still inclosed within its sheath, progressive motion being unimpeded by the latter.

Periodicity.—Filaria philippinensis presents no periodicity as regards the time of its appearance in the peripheral blood or the number of embryos present at any one time. We have made extensive observations regarding periodicity in all five cases of infection with this filaria and can state conclusively that it can be found in the peripheral blood at all hours of the day and night and in practically the same numbers at all hours. The following table, which gives the results of examinations made every three hours during the twenty-four, demonstrates the lack of periodicity in *Filaria philippinensis:*

Case number 1.

Number in individual counts.	*Average per day and night.*
7 a. m., 3 filariæ in 3 blood smears.	
10 a. m., 2 filariæ in 3 blood smears.	
1 p. m., 2 filariæ in 3 blood smears.	7 a. m. to 7 p. m., 8 filariæ in 12 smears.
4 p. m., 1 filaria in 3 blood smears.	
7 p. m., 2 filariæ in 3 blood smears.	7 p. m. to 7 a. m., 8 filariæ in 13 smears.
10 p. m., 1 filaria in 3 blood smears.	
1 a. m., 3 filariæ in 4 blood smears.	
4 a. m., 2 filariæ in 3 blood smears.	

Case number 2.

Number in individual counts.	*Average per day and night.*
7 a. m., 5 filariæ in 3 blood smears.	
10 a. m., 1 filaria in 4 blood smears.	
1 p. m., 1 filaria in 3 blood smears.	7 a. m. to 7 p. m., 8 filariæ in 13 smears.
4 p. m., 1 filaria in 3 blood smears.	
7 p. m., 2 filariæ in 3 blood smears.	7 p. m. to 7 a. m., 8 filariæ in 13 smears.
10 p. m., 3 filariæ in 3 blood smears.	
1 a. m., 0 filaria in 3 blood smears.	
4 a. m., 3 filariæ in 4 blood smears.	

Case number 3.

Number in individual counts.	*Average per day and night.*
7 a. m., 2 filariæ in 3 blood smears.	
10 a. m., 1 filaria in 4 blood smears.	
1 p. m., 3 filariæ in 3 blood smears.	7 a. m. to 7 p. m., 8 filariæ in 3 smears.
4 p. m., 2 filariæ in 3 blood smears.	
7 p. m., 4 filariæ in 3 blood smears.	7 p. m. to 7 a. m., 10 filariæ in 13 smears.
10 p. m., 0 filaria in 3 blood smears.	
1 a. m., 5 filariæ in 4 blood smears.	
4 a. m., 1 filaria in 3 blood smears.	

A study of the above table will show that at no definite time during the twenty-four hours can the filariæ be said to be numerous enough to be characteristic of that time, and that no difference exists in the number observed in the peripheral blood during day and night. This fact, together with the presence of a sheath in *Filaria philippinensis,* serves to differentiate it from any other filaria which has been described as infecting man. It should be noted that it occurs in small numbers at all times and this has been a prominent feature in all of our observations. It appears to us that this is a point of considerable diagnostic importance, for in all five of the cases practically the same number of filariæ was observed and that number was always a small one when compared with the immense quantities of *Filaria nocturna, Filaria diurna,* and *Filaria perstans* Manson which are commonly found in cases infected by the last three organisms.

Pathogenicity.—In none of the cases did we observe any symptoms or pathological lesions which we could feel justified in considering as being due to the presence of the filaria. As stated in our original communication, one patient had a history of having suffered from chyluria, but during the time he was under our observation he presented no symptoms; one man had a periostitis while under observation and one, amœbic dysentery. An examination of the fæces of three of the four Scouts demonstrated an interesting series of combined infections due to animal parasites. Of the three men, one had a combined infection with *Filaria philippinensis, Anchylostoma duodenalis* and *Trichocephalus dispar;* the second, with *Filaria philippinensis, Entamœba dysenteriæ, Anchylostoma duodenalis* and *Trichocephalus dispar,* and the third with *Filaria philippinensis* and *Anchylostoma duodenalis.*[2]

THE DEPELOPMENT OF FILARIA PHILIPPINENSIS WITHIN CULEX FATIGANS.

We have made numerous examinations of mosquitoes which were infected by biting patients who harbored embryos of *Filaria philippinensis,* most of the insects so infected being *Culex fatigans* Wied, some few being *Stegomyiæ.* In our examinations we have not found that the filariæ undergo any development in *Stegomyiæ,* and think it probable that they do not, for, while our observations on this point are not sufficiently numerous to justify us in making positive statements, it is asserted by Daniels that *Filaria nocturna* will not develop in *Stegomyia.*

However, in *Culex fatigans* Wied, we have been able to trace the complete development of the filaria up to the time that it becomes lodged in the mosquito's labium and is ready to infect the next person bitten by the insect. The stages of this development are well shown in the accompanying photomicrographs and in numerous camera-lucida drawings, and may be quite as well, or better, studied from them as from the written description which here follows:

One of the earliest observations to be made in studying infected mosquitoes is one relating to the number of filariæ ingested. In some manner, concerning which it is possible to construct interesting hypotheses, the mosquito manages to get from the body of the patient 40 to 50 or more times as many filariæ as it is possible for us to obtain in a similar amount of blood from a needle prick. Thus, in good thick smears under 22-millimeter (⅞-inch) cover-glasses, we will usually find but one or two, and oftentimes no filariæ. In the blood from the stomach of a mosquito which has recently bitten, the amount of blood usually appears to be less than in these thick cover-slip preparations, but it is nearly always possible to find from 40 to 80 filariæ, and it has occurred to us that this fact might have a practical value in examining cases of suspected filariasis in which the parasites are so few in number as readily to be missed. It might be of use in revealing embryos in the blood of cases of elephantiasis, where the filariæ, though believed to be present, are seldom found. We have not had an opportunity to put our suggestion into practice except on our last four cases of known filariasis, in all of which the result has been as stated.

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[2] In view of the recent finding by Lieutenant Clarence L. Cole, Medical Department, United States Army, of *Necator americanus,* in natives of these Islands, it is possible that our cases were infected with it, as the ova were not measured.

Once in the stomach of the mosquito, the filariæ continue their active motion, which is more uniformly progressive than in the fresh blood, for several hours (the exact length of time we are unable to state) until they discard their sheaths and pierce the stomach wall, entering the body cavity.

Stages of growth of Filaria philippinensis within Culex fatigans Wied.—It is necessary to state here that the various stages of growth are not passed through in uniform intervals of time but that the periods mentioned below, as well as the measurements given, are thought merely to represent averages. Thus, embryos free from their sheaths may be found in the tissues outside of the stomach, while others ingested at the same time are still struggling in the stomach to free themselves. Likewise, at the eighth day we have, in a heavily infected mosquito, seen some filariæ which appeared to have undergone fully twice as great a degree of development as others, although we knew that they had all been ingested at one feeding.

This preliminary being understood, we may say that in twenty-four hours after ingestion by the mosquito the filariæ have escaped from their sheaths and pierced the stomach wall, being found free in the abdominal cavity. Here they are sluggish in motion, unchanged as to size, but have a more granular appearance. The V spots may often still be distinguished, but the central viscus can not. It is not uncommon to find some of these worms dead and we think that the mortality among them is high at all stages of their development, but more particularly so during the early ones. It is unusual to find more than four or six filariæ which undergo the complete cycle of development within the mosquito at one time, while on the other hand only one may remain, and most frequently none are to be observed in the mosquito at the end of ten days or two weeks. Once we encountered fifty-one filariæ in a mosquito's thorax on the eighth day, but we have in no other instance found anything like these numbers after the second.

By the third day, possibly earlier at times, the filariæ have left the abdominal cavity and are to be seen among the thoracic muscles of the mosquito, where they show very sluggish motion and are observed to have undergone a very marked change in morphology.

The length has decreased from 0.32 to 0.21 millimeter; the breadth has increased from 0.0065 to 0.01 millimeter; all parts of the body have participated in the increase in width except the tip of the tail—that is, the part of the tail back of the sudden diminution described in our first paper. This part has not enlarged in size, and, as will be seen, it disappears at a later period, thus fulfilling the prediction concerning it which we made in our first publication. The posterior V spot is still visible and is manifestly increased in size. The body presents a granular appearance and the central viscus has disappeared. (See figs. 2, 3, 19, and 20.)

At the end of six days the filariæ are still as short as at three days, but they have undergone a further increase in breadth which at this stage varies from 0.014 millimeter near the anterior end to 0.027 millimeter near the posterior, the average being about 0.02 millimeter. The tip of the tail is still present and still unenlarged and now presents a "pigtail-like" appearance in its attachment to the more bulky body. The latter still has a granular aspect but some cell structure can be made out and the alimentary canal outlined. The posterior V spot, as such, has disappeared, but its place is occupied by a rudimentary anus. The motion of the worm is still sluggish, being about the same as it is at the third day. (See figs. 4, 5, and 21.)

On the eighth day we find the filaria increased in length and in breadth; the "pigtail" portion of the tail in most instances, though not in all, has disappeared; the alimentary canal can be traced the whole length of the worm. The length averages about 0.6 millimeter, the breadth 0.035 millimeter; motion is sluggish but the filaria is possibly a little more active than it was at the sixth day. (See fig. 22.)

From the eighth to the eleventh day the worm undergoes a more rapid development than in any other similar period and at the expiration of this time is found to have attained a length of from 1.2 to 1.6 millimeters and a width varying from 0.04 down to 0.02 millimeter, the width being less in the longer worms. (See figs. 6, 7, 8, and 23.)

Some worms at this time show as complete a development as we have ever seen and apparently have no other change to undergo except the lengthening and narrowing, a change of form rather than of size, necessary to enable them to enter the labium. The head is truncated, the mouth a terminal, crater-like, circular opening, forming the base of a conical cavity; no lips are visible. Nothing recognizable as the analogue of the anterior V spot is distinguishable. The intestinal canal is well developed, the œsophagus straight and broad, the intestine narrower and much coiled, particularly at a point corresponding in situation to that of the spiral viscus in the blood embryo. The anus is a slit, directed from within outward and backward (see fig. 17); the intestine, apparently, does not terminate at the anus but continues to the end of the body. The tail is tipped with three well-defined papillæ; this number is probably constant, although only two are usually visible in profile.

By the eleventh or twelfth day some of the filariæ may be found in the mosquito's head, by the fourteenth or fifteenth they are encountered in the labium, having completed their development in the insect, so far as we have been able to observe. At this stage the filariæ are found within the labium, the fleshy underlip of the insect, where they lie side by side, with their heads directed toward the labella. We have observed as many as four within the labium, and it is probable that as a rule they occur in this situation in pairs. (See figs. 13, 14, 15, and 16.)

As our work has been done by teasing preparations and not by sectioning, we are unable to state exactly where the filariæ lie in the head or how they get into the labium. However, the fact that they do reach that situation is beautifully illustrated in the photomicrographs, which show a fragment of the labium with the tails of the filariæ protruding from the proximal end. This preparation was made on the sixteenth day after the mosquito had bitten the filariasis patient.

The worm, at this stage, has diminished markedly in breadth, the average measurement for this dimension being not more than 0.02 millimeter, while individuals have been measured in which it was only 0.015 millimeter. The former figure, however, applies in most instances. The length varies greatly and we have measured specimens which range from 1.236 to 2.20 millimeters. We can say from our observations that the worm when found in the mosquito's labium may average 1.8 millimeters in length, but that others in the same situation and in the same mosquito, actively motile and apparently ready to pass to another host, may be as short as 1.24 or 1.27 millimeters. Two worms taken from the mosquito's head upon the eleventh day gave 1.496 and 1.630 millimeters, respectively, and this variation in length is noticed in individual worms after the very earliest stages in the mosquito. We have thought that the long and short groups may represent the sexes, but have no other evidence to offer in support of such a supposition. (See figs. 10, 11, 24, and 25.)

At this stage of development the alimentary tract is well defined and extends from the mouth to the anus, which is well marked; it possesses some strength, as is shown in fig. 9, which represents a worm broken by the attempts made to dissect it from the labium. Here the fragments are held together by the intact cellular tube forming the intestinal canal. The anus is well developed and is easily seen, presenting the same appearance as it does at the eleventh day of development.

Another point easily determined at this time is that the post-anal papillæ are three in number. Annett, Dutton, and Elliot state that

Filaria nocturna has four of these papillæ and we have carefully looked for four on this filaria. There is no difficulty in making out three in *Filaria philippinensis,* but in no instance have we seen four. When the end of the tail is facing the observer it will be seen that the three papillæ are arranged in a triangular manner and are apparently curved inward. (See figs. 12 and 26.)

In most spicimens an appearance suggestive of two narrow tubes, running in general parallel with the alimentary canal, one on either side, may be seen. These tubes we think probably represent the future generative organs, but we are unwilling to make positive statement either as to their constant presence or their significance. They, or certain masses of cells extend posteriorly to the anus, apparently clear back to the terminal knobs or papillæ, and in at least one specimen their terminations appeared to constitute the papillæ.

Some of the filariæ from the head and labium are very actively motile; others show no motion, but as these presented no evidence of degeneration we think that they were probably killed by the chloroform used in destroying the mosquitoes.

The following table gives the principal differential features between the embryos of *Filaria philippinensis* and of other known filariæ, as they occur in the blood of man:

Name.	Length.	Breadth.	Sheath.	Head.	Tail.	Anterior V spot.
	mm.	*mm.*				
F. philippinensis sp. nov.	0.32	0.0065	Present, tight.	Serrated retractile band and spicule.	Pointed, abruptly attenuated.	Present.
F. nocturna Manson.	0.30	0.0075	Present, loose.	Six lips	Pointed	Do.
F. diurna Manson.	0.30	0.0075	do	do	do	Do.
F. perstans Manson.	0.20	0.0045	Absent	Retractile fang	Blunt	Do.
F. demarquayi Manson.	0.20	0.005	do	Spine	Pointed	Do.
F. ozzardi Manson.	0.21	0.0051	do	(?)	do	Absent.
F. magalhaesi R. Blanchard.	0.33	0.005	do	Unarmed	do	Do.
F. volvulus Leuckart.	0.30	0.005	do	Rounded	do	Present.
F. tanaguchii Tan.	0.295	0.007	do	Blunt	do	Absent.
F. ? (*Tanaguchii*)	0.164	0.008	Present	do	Conical	Do.
F. gigas Prout	Much longer and thicker than any of the above.		Absent	(?)	Blunt	(?)

Name.	Central viscus.	Posterior V spot.	Movement.	Periodicity.	Adult.
F. philippinensis sp. nov.	A spiral tube or cylinder.	Present; also a papilla.	Lashing and progressive.	None	Not found.
F. nocturna Manson.	Granular mass	Present	Lashing	Nocturnal	*F. bancrofti.*
F. diurna Manson.	Absent	do	do	Diurnal	Not found.
F. perstans Manson.	do	Absent	Lashing and progressive.	None	*F. perstans.*
F. demarquayi Manson.	(?)	(?)	Progressive	do	*F. demarquayi.*
F. ozzardi Manson.	Absent	Absent	do	do	*F. ozzardi.*
F. magalhaesi R. Blanchard.	do	do	(?)	(?)	*F. magalhaesi.*
F. volvolus Leuckart.	do	do	(?)	(?)	*F. volvolus.*
F. tanaguchii Tan.	Granular streak from mouth to tail.	do	Progressive	None	*F. taniguchii.*
F. ? (*Tanaguchii*)	Absent	do	Lashing	(?)	Not found.
F. gigas Prout	(?)	(?)	(?)	(?)	Do.

CONCLUSION.

Briefly summarized, the history of the development of *Filaria philippinensis* within the mosquito, *Culex fatigans* Wied., is as follows: In from fourteen to fifteen days the development is complete and the filaria has passed into the labium of the mosquito; the sheath of the embryo is lost in the stomach, and the worm then penetrates the stomach-wall and reaches the muscles of the thorax where most of the developmental changes occur; during this period of time the filaria has increased in length from 0.32 millimeter to as much as 2.20 millimeters, and in breadth from 0.0065 to 0.02 millimeter; it has developed a well-marked intestinal canal, divided into oesophagus and intestine, a well-defined anus and three papillæ which are situated at the end of the tail; the mouth appears to be simply a circular cavity having no distinct lips. Development so far as the morphology of the worm indicates, appears to be complete at about the eleventh day, the only changes occurring after that being a lengthening and narrowing of the filaria, which enables it to enter the labium of the mosquito.

In concluding, we desire to express our appreciation of the assistance given us by Mr. Charles S. Banks, entomologist of the Biological Laboratory, Bureau of Science, and Mr. Charles Martin, photographer of the Bureau of Science.

ILLUSTRATIONS.

PLATE I.

FIG. 1. *Filaria philippinensis* in freshly drawn blood.
2. *Filaria philippinensis* three days in *C. fatigans.* × 115.
3. *Filaria philippinensis* three days in *C. fatigans.* × 229.

PLATE II.

FIG. 4. *Filaria philippinensis* six days in *C. fatigans.* × 92.
5. *Filaria philippinensis* six days in *C. fatigans.* × 206.
6. *Filaria philippinensis* eleven days in *C. fatigans.* × 76.
7. Tail of *Filaria philippinensis* eleven days in *C. fatigans.* × 183.

PLATE III.

FIG. 8. Head of *Filaria philippinensis* eleven days in *C. fatigans.* × 275.
9. Broken *Filaria philippinensis* fifteen and one-half days in *C. fatigans.* × 115. Showing strength of alimentary canal.
10. *Filaria philippinensis* fifteen and one-half days in *C. fatigans.* × 115. Showing elongation and narrowing in labium.

PLATE IV.

FIG. 11. *Filaria philippinensis* fifteen and one-half days in *C. fatigans.* × 220. Showing alimentary canal. A portion of the alimentary canal has been extruded by pressure.
12. Tail of *Filaria philippinensis* fifteen and one-half days in *C. fatigans.* × 275. Showing three papillæ.
13. *Filaria philippinensis* fifteen and one-half days in *C. fatigans.* × 92. In labium.
14. *Filaria philippinensis* fifteen and one-half days in *C. fatigans.* × 220. In labium.
15. *Filaria philippinensis* fifteen and one-half days in *C. fatigans.* × 86. In labium.

PLATE V.

FIG. 16. *Filaria philippinensis* fifteen and one-half days in *C. fatigans.* × 240. In labium.
17. *Filaria philippinensis* eleven days in *C. fatigans,* showing anus.

PLATE VI.

FIG. 18. *Filaria philippinensis* in freshly drawn blood. Killed and drawn by the aid of camera-lucida at once. × 330.
19. *Filaria philippinensis* in stomach of *C. fatigans* six to twelve hours after ingestion. Sheath discarded. × 330.

20. *Filaria philippinensis* in thorax of *C. fatigans* three days after ingestion. Note size of worm and size of post V spot. × 330.
21. *Filaria philippinensis* in thorax-of *C. fatigans* six days after ingestion. Note part of contents extruded through V spot. × 330.
22. *Filaria philippinensis* in thorax eight days after ingestion. × 63 and × 330.

PLATE VII.

FIG. 23. *Filaria philippinensis* in thorax eleven days after ingestion. × 63 and × 330.
24. *Filaria philippinensis* in labium fifteen and one-half days after ingestion. × 63 and 330.
25. *Filaria philippinensis* in labium fifteen and one-half days after ingestion. × 63 and 330.
26. *Filaria philippinensis* in labium fifteen and one-half days after ingestion. × 63 and 330.

Note. In Figs. 22 to 26, the entire filaria is represented at A, the tail at B and the head at C.

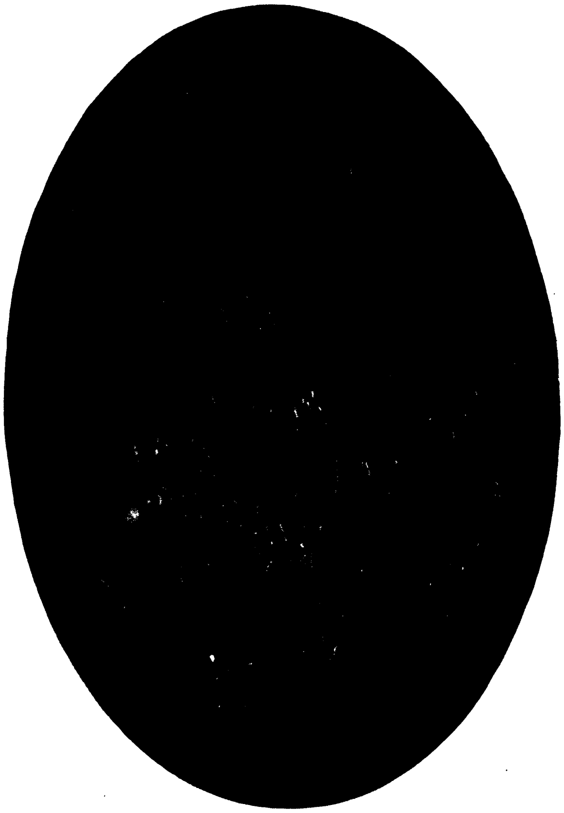


FIG. 1.

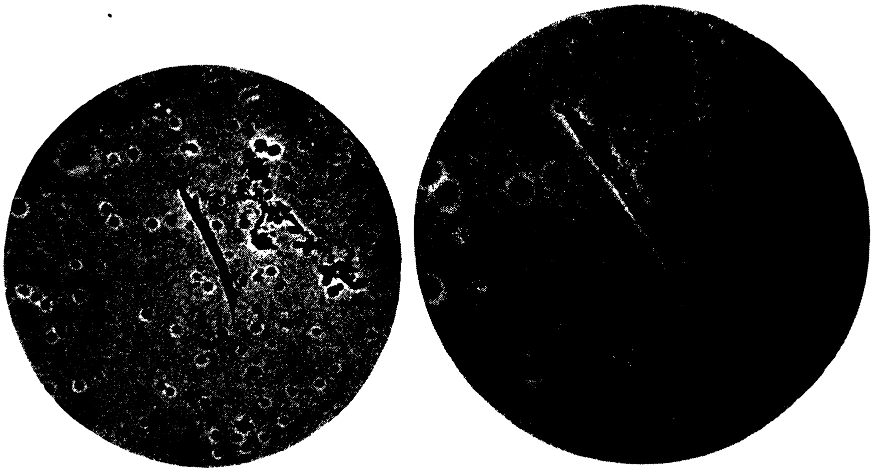


FIG. 2. FIG. 3.

PLATE I.

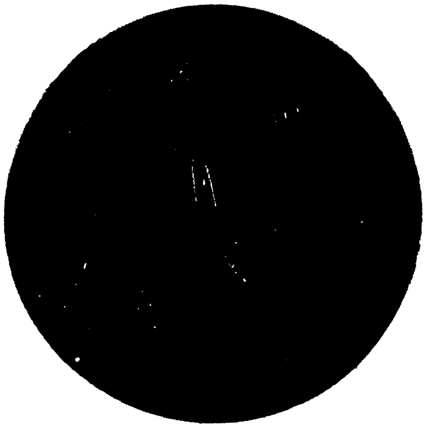
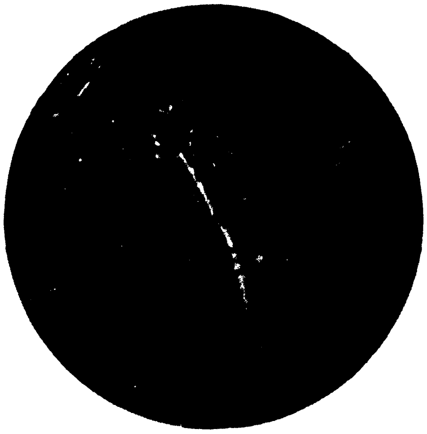


FIG. 4.



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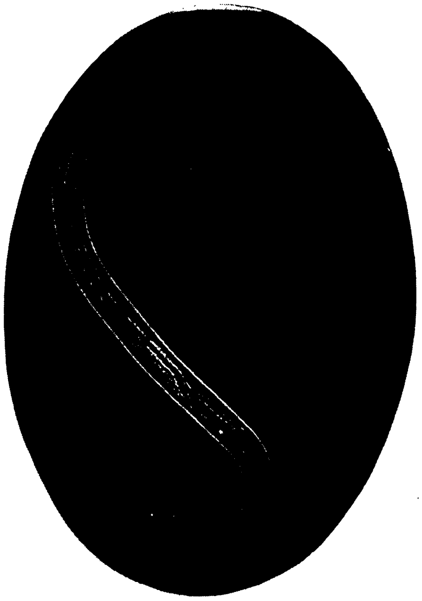

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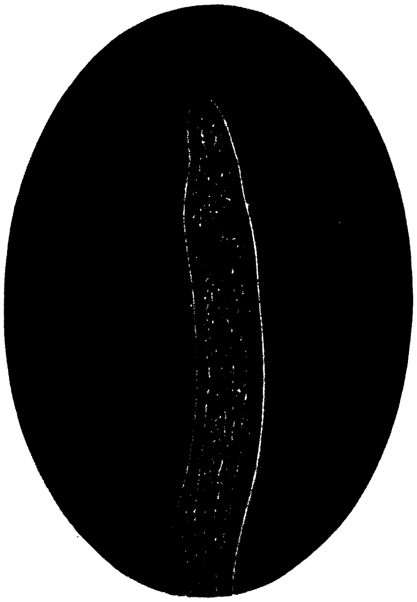


FIG. 7.

PLATE II.

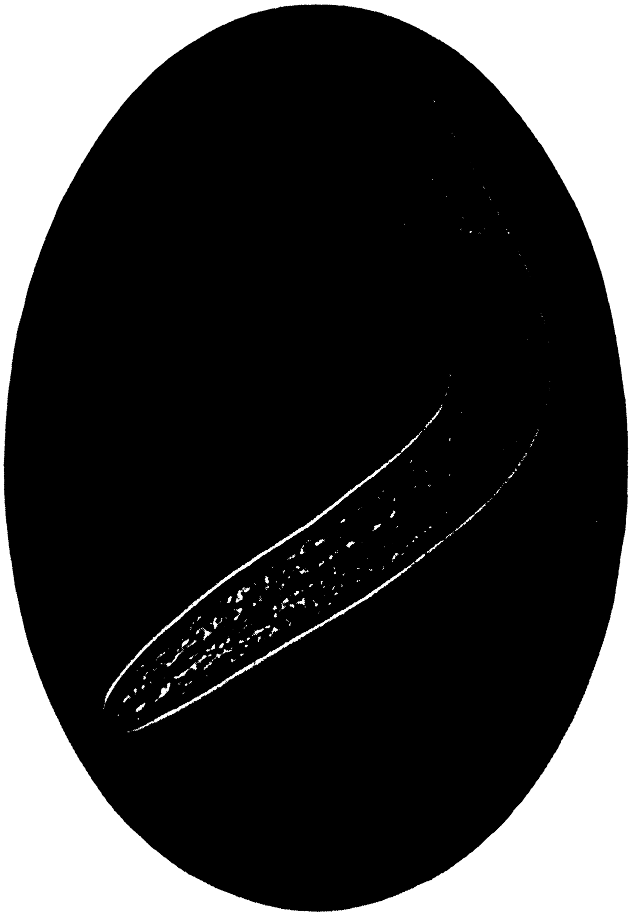


FIG. 8.

FIG. 10.

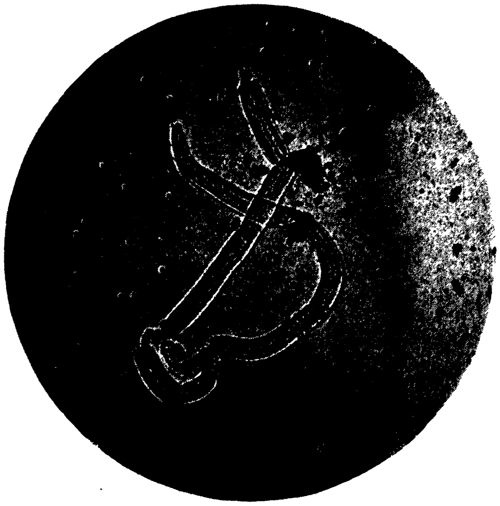


FIG. 9.

PLATE III.

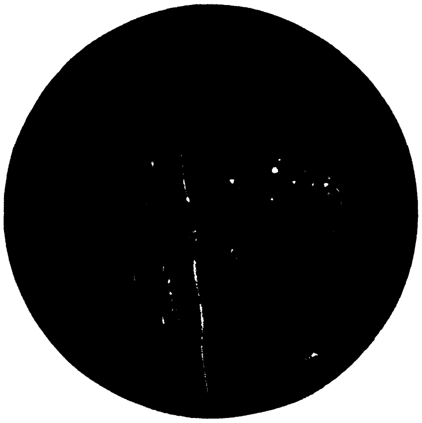


FIG. 11.

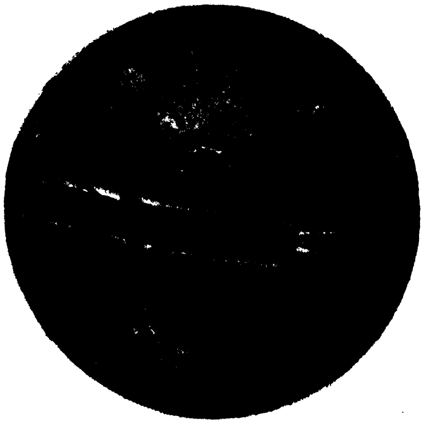


FIG. 13.

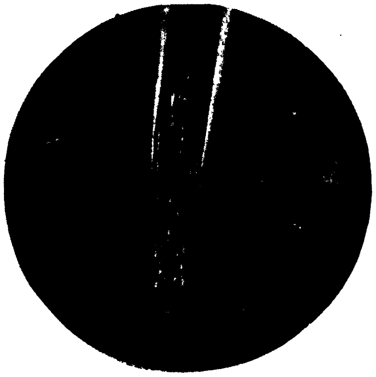
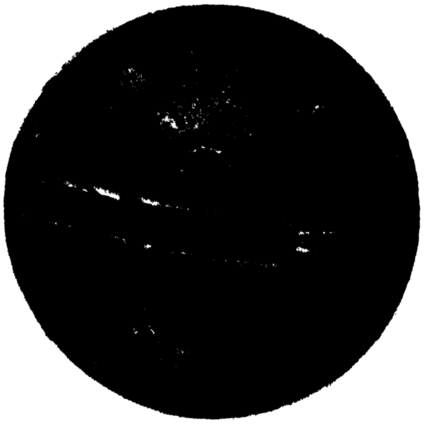


FIG. 12.



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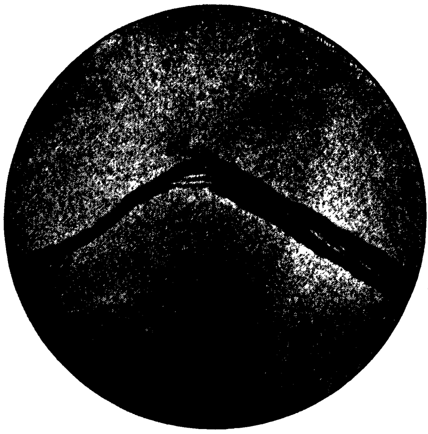

FIG. 15.

PLATE IV.

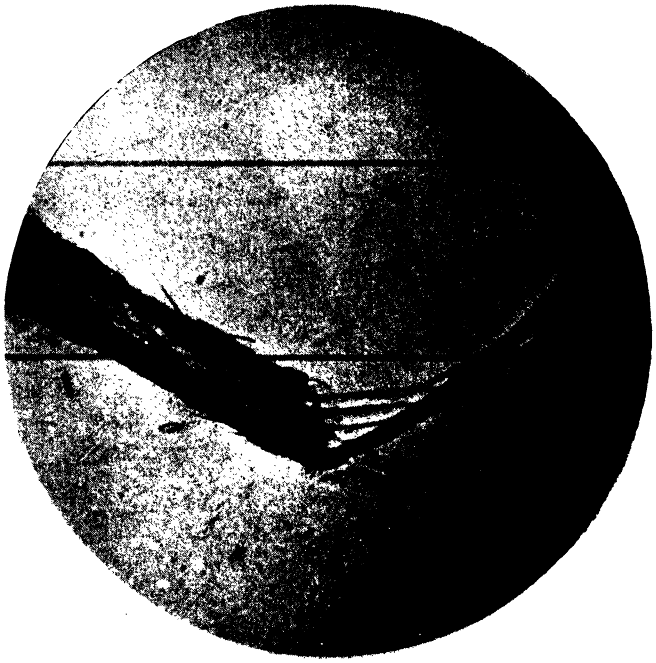

FIG. 16.

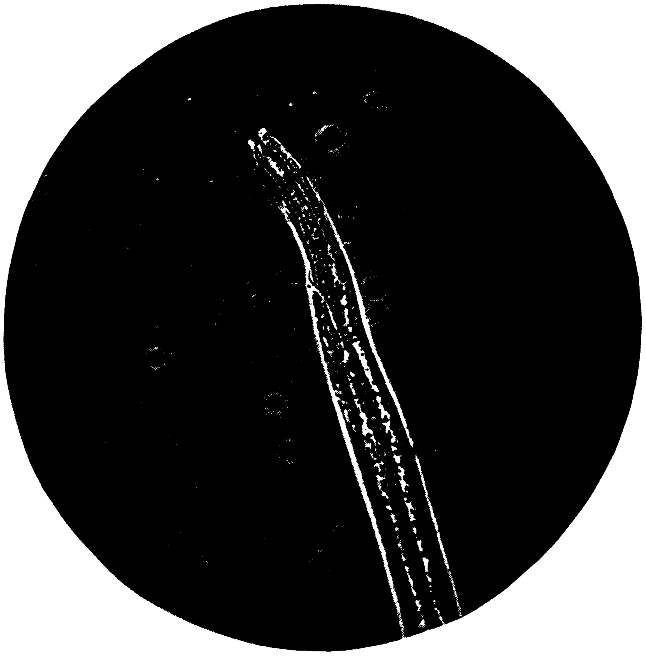

FIG. 17.

PLATE V.

Fig 18

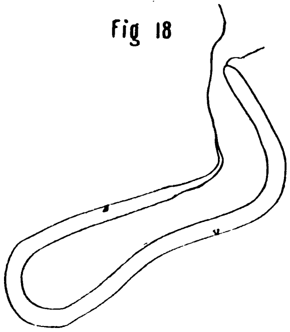

Fig. 19

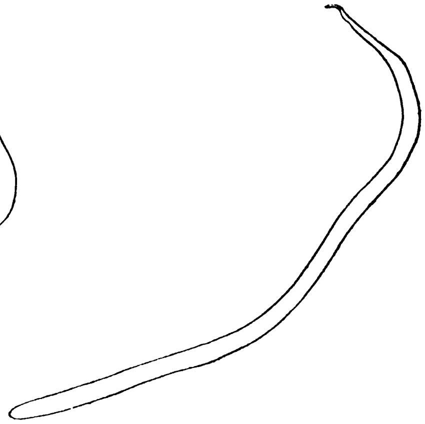

Fig. 21

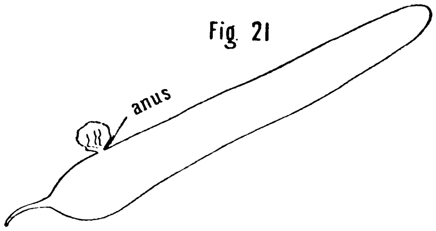

Fig. 20

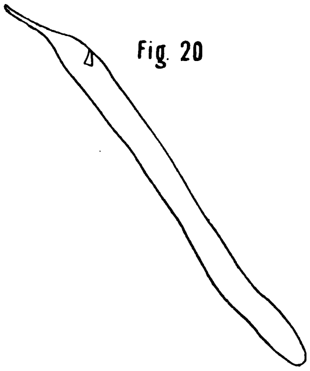

Fig. 22

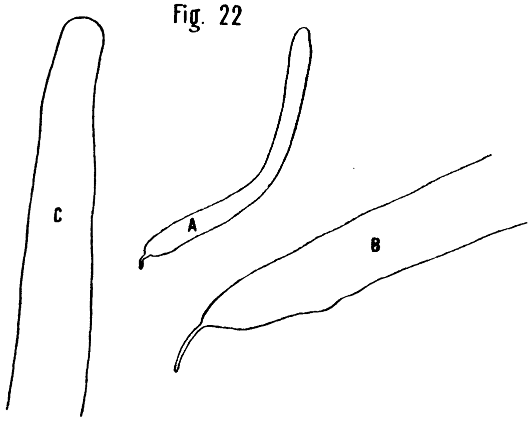

PLATE VI.

Fig. 23

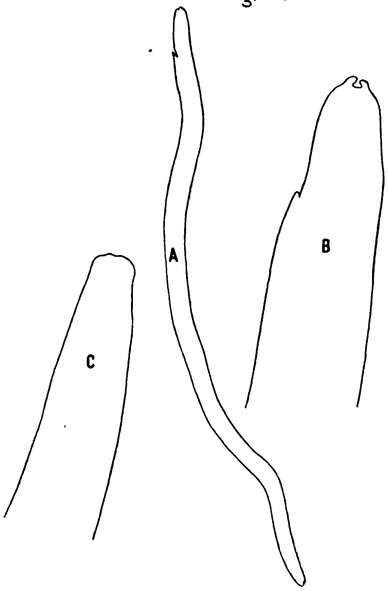

Fig. 24

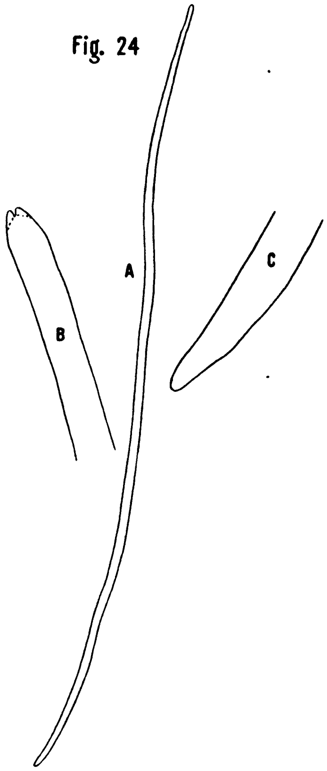

Fig. 25

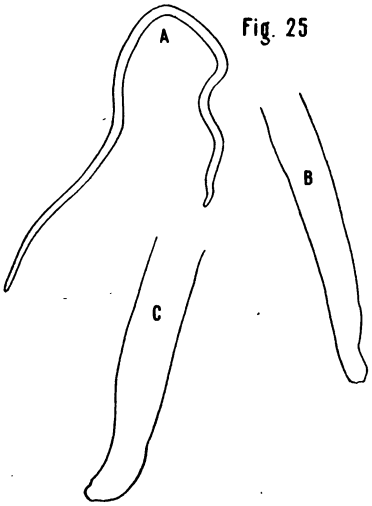

Fig. 26

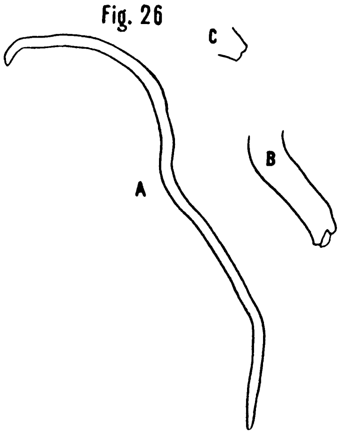

Plate VII.

PARAGONIMIASIS IN THE PHILIPPINE ISLANDS.

By W. E. MUSGRAVE.
(*From the Biological Laboratory, Bureau of Science.*)

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I. INTRODUCTION.

Trematode infections, next to the protozoan diseases, are the most important parasitic conditions which must be met by medical men in the Philippine Islands.

The order *Malacocotylea* is a very large one, containing many species, but of these only a comparatively small number are known to infect man; those which are parasitic for human beings, compiled from Stiles's publication, are shown in Table No. 1, page 31. They represent 4 families, 10 genera or collective groups, and 15 species. Of this number only about 8 species are sufficiently common in man to be of great importance. All flukes are considered to be disease producers, regardless of the stage of the life cycle of the parasite or of the nature of the host in which they are found. The adult parasites with a very few exceptions, whether in man or other animals, are found only in diseased tissues, and the miracidia and cercaria may produce disease of snails and other hosts in which they may be living.

So far as I have been able to determine, only two cases of trematode infection have previously been reported from the Philippine Islands. One of these was a case of infection with *Opisthorchis sinensis* Cobbold, reported by Dr. Mallory at the last annual meeting of the Philippine Islands Medical Association, in which the diagnosis of the egg was made by Dr. Strong; the other was one of schistosomiasis by Dr. Woolley.

The following paper is the first of a series to be published during the year on material which is already available, as follows:

One case of infection with *Opisthorchis noverca* (?) Braun, in a Chinaman; 2 cases with autopsies, in which the infecting flukes, though not thoroughly identified, are probably *Fasciola gigantica* Cobbold and *F. hepatica* Linn., respectively; 8 cases infected with *Schistosoma japonicum* Katsurada, with 2 autopsies; 18 with *Opisthorchis sinensis* Cobbold, with 1 autopsy, and 17 with *Paragonimus westermanii* Kerb., with 8 autopsies; the total being 46 cases in human beings. I have also studied quite a large series of infections in the lower animals.

These reports appear in monographic form because the observations have included too many points, not only about the parasites but also particularly about the diseases caused by them, to allow of a satisfactory presentation of the findings in any other manner.

I wish to express my thanks to Dr. Edwin C. Shattuck, resident physician to Bilibid Prison, for many courtesies and much assistance in the observations on the clinical material, much of which is from his service.

II. DEFINITION.

Paragonimiasis is a chronic or subacute, general or local infection, with a species of the genus *Paragonimus,* only one species of which genus, *P. westermanii,* is known to infect man. The disease is characterized anatomically by the production of peculiar, bluish, slate-colored, necrotic, cystic lesions with rather dense, fibrous walls which contain a material resembling anchovy sauce and usually, but not always, the eggs or adult *Paragonimus.* Other types of lesions are often present and many different organs may be involved. The most frequent are bronchopneumonia and bronchiectasis; cystic abscess of the brain, spleen, omentum, pancreas, muscles, lymphatic glands, and skin; and infiltration, ulceration, and abscess formation in the intestine.

The symptomatology in general is that of a chronic inflammatory process, and special clinical manifestations are due to the involvement of special organs which give rise to such symptoms as cough, hæmoptysis, pleuritic pains, diarrhœa and abdominal soreness. The life history of the parasite and the exact mode of infection by it are not known.

III. NOMENCLATURE.

A. SYNONYMS.

The following may be mentioned among the many names by which this disease has been known: Parasitical hæmoptysis, parasitic hæmoptysis gregarinosis pulmonis, pulmonary distomatosis, pulmonary distomiasis, endemic hæmoptysis, and paragonimiasis. Parasitical hæmoptysis is the most extensively used of these terms and at the time of its introduction it answered very well, since formerly the distribution of the parasites throughout the body was regarded as limited and it was not known that such extensive infection existed as has recently been described.

All of these names, except paragonimiasis, are objectionable because none of them are accurate, specific or definite, and their number leads to a confusion which can only be overcome by the adoption of a term which fulfills all the requirements of nomenclature. Paragonimiasis is such a name. It is in line with modern nomenclature in parasitic infections and is applicable to all types of the diseases, whether they are found in man or in animals.

This term was introduced by Stiles and Hassall and has been adopted by Mense in his new Handbook of Tropical Medicine. It is the only name for the infection which will be used in this report and, owing to the extensive distribution of the parasite in man and the consequent logical variation in symptomatology, I shall recognize the following types of the disease in their appropriate places: General paragonimiasis, thoracic or pulmonary paragonimiasis, cerebral paragonimiasis, and abdominal paragonimiasis.

IV. BRIEF HISTORY.

A. GENERAL.

Paragonimus westermanii was discovered by Kerbert in 1878 in the lungs of a royal Bengal tiger and was by him named *Distoma westermanii.* In 1879 Ringer found the parasites in a native of Formosa. Cobbold (1880) named the parasite *Distoma ringeri.*

Baelz (1880), who was working in Japan, introduced the name *parasitic hæmoptysis* (*parasitäre hämoptoë*) for the disease and discussed the parasite under the designations *Gregarina pulmonum* and *Gregarina fusca;* he further used *gregarinosis pulmonum* as a synonym for the disease. Baelz gave further valuable contributions on the subject in 1883 and 1901. In 1883 he adopted the name *Distoma pulmonale* for the parasite

Suga (1881) found the infection in man in Japan, and in 1883 described the parasite as *Distomum pulmonis.*

Manson made valuable original contributions to the subject in 1881, 1883, 1889, and 1893. He proposed *parasitical hæmoptysis* and *endemic hæmoptysis* as names for the disease and designated the parasite as *Distoma ringeri* and *Distoma pulmonale.* Further information of value has been furnished by Taylor (1884), Leuckart (1889), Yamagiwa (1890 and 1892), Stiles (1894, 1901, and 1904), Stiles and Hassall (1900), Ward (1893 and 1895), Katsurada (1898 and 1900), Miura (1889), and by a number of Japanese writers whose articles are not available. Prominent among these are Otani, Taniguchi, Majima, Miyake, and Matsui.

The landmarks in the history of this parasite and the diseases caused by it are as follows:

The discovery of the parasite by Kerbert (1878); the discovery of the parasite in human beings by Ringer (1879); the establishment of the relation of the parasite to the disease in man by Suga, Baelz, and Manson; the discovery of the more extensive distribution of the parasite and its location in the brain, producing Jacksonian epilepsy, by Otani and Yamagiwa; its discovery in the eye and lids by Taniguchi, Miyake, and Matsui; the establishment of the zoölogical position of the parasite by Stiles and Looss; the discovery of the infection in various animals, i. e., in the tiger by Kerbert, 1878; the cat by Ward, 1894; the dog by Railliet, 1890, and the hog by Stiles, 1899; the discovery of the parasite in the United States by Ward, and the elucidation of the pathology and symptomatology of the disease by Manson, Baelz, Miura, and Katsurada.

B. IN THE PHILIPPINE ISLANDS.

Because of the geographic position of the Archipelago and the close commercial and social intercourse between these Islands and Japan and China, it has repeatedly been predicted that fluke infections would be found here. Notwithstanding this expectation and the large amount of clinical and anatomical work which has been done in the Philippines, this is the first report of infections with *P. westermanii* Kerb. in the Archipelago, either occurring in natives or foreigners. As was mentioned in the introduction, there have been but two previous reports, of infection by trematodes of any species in the Philippine Islands, these being of single cases, one of *opisthorchiasis* and the other of *schistosomiasis.*

I encountered my first case of paragonimiasis early in 1906 and since that time have seen 16 others. The discovery of this number of cases in less than one year by one observer does not, in my opinion, indicate any exacerbation of the infection nor any change in the class of available material, but is due to a closer observation and study of that which is at hand. Since becoming interested in this class of diseases and familiar with the appearance of the various lesions, I am confident that similar material has passed through my hands unrecognized many times during the last seven years, and identical experiences have probably occured with others. It may safely be stated, to judge from the wide local distribution of my cases, that trematode infections are fairly common among the natives of these Islands and that the infection has been endemic here for many years.

V. MATERIAL.

The material upon which this report is based consists of 17 cases with 8 autopsies. Abstracts from the protocols follow and the summaries of the findings and the analyses of the lesions and symptoms will be taken up under the appropriate headings furnished in the outline.

Case No. 1 (30918).—Paragonimiasis and amœbiasis of the lungs, pleuræ, diaphragm, omentum, peritoneum, spleen, liver, intestine, lymphatics, prostate, bladder and abdominal wall; death; autopsy.

The patient, a Filipino prisoner, was under observation in hospital for one week, suffering with high fever, 30° to 40°, severe cough, pains in the chest, and some dyspnœa. There was severe head-ache, mild delirium at times and insomnia. The bowels were constipated, the abdominal wall rigid, and tenderness was complained of over the entire abdomen. A blood examination showed nothing abnormal except a moderate polymorphonuclear leucocytosis. The sputum was negative for tubercle bacilli, but on the morning before death a few trematode eggs were found. The stool contained many amœbæ, blood cells, and mucus and a few eggs similar to those in the sputum.

AUTOPSY two hours after death: Emaciation and anæmia marked, severe conjunctivitis of left eye; old, bluish-colored ulcer in the right axilla, which on section connects with broken-down lymphatic glands. The eggs of *P. westermanii* are found in this ulcer. The superficial lymphatics are all moderately enlarged and those in the groins markedly so, and this enlarged chain extends down into Scarpa's triangle. The scrotum is enlarged and on section the epididymis contains *Paragonimus* cysts which apparently are continuous through the inguinal canal to the abdominal cavity.

The *subcutaneous tissue* is scanty and dry, the muscles soft, pale and coarse. A small *Paragonimus* cyst is observed in the left pectoralis major and another in the abdominal wall just above the symphisis. Upon opening the chest a number of the small bluish *Paragonimus* cysts are found on the under surface of the sternum and on the exposed pleuræ and pericardial membranes. Both *pleural cavities* contain a considerable quantity of fluid, filled with stringy masses and flocculi which settle to the bottom upon standing. The *pleuræ*, particularly the visceral layers, are much thickened. The pleural surfaces are covered with many of the cysts and even external to the parietal pleuræ these lesions are found. There are several small areas of chronic obliterative pleuritis and *Paragonimus* lesions are numerous in the fibrous tissue forming the adhesions. Both *lungs* show a moderate bronchitis, particularly of the larger tubes, but no eggs or parasites are found in the bronchi and no definite bronchiectatic cavities can be located. There are numerous areas of bronchopneumonia throughout both lungs, the largest near the pleural surfaces; many of these pneumonic areas are not to be distinguished from such lesions in other diseases of bacterial origin, while others, on section, have a necrotic center containing material like anchovy sauce and often, but not always, adult parasites or eggs of *P. westermanii.* In addition to these pneumonic areas, both lungs contain many of the typical bluish *Paragonimus* cysts from 0.005 to 0.01 in diameter.

The *pericardial cavity contains* a small amount of a slightly cloudy fluid. The serous surface is dull in appearance and slightly adherent over the right heart; showing through the visceral pericardium are a number of irregular, slightly brownish, granular appearing areas similar to those described in case No. 2 (see Pl. 1). Eggs are found in these areas but none in the pericardial fluid. The *heart* is of normal size and free from valvulitis or other marked organic change. The muscle is a little brown and coarse, but except just under the pericardium no gross lesions are seen.

The *abdominal cavity* presents a most striking picture, all visible tissues being matted together and studded with the bluish-colored *Paragonimus* cystic abscesses. In places where adhesions have been separated, for instance, between the bowel surfaces or omentum and the abdominal wall, an area of bluish color of equal size to the adhesions is left, and often small, necrotic areas with eggs and parasites are exposed. Everywhere there is a moderate, subacute peritonitis, light, dry, dirty-colored adhesions binding all the organs. Both the abdominal and pleural surfaces of the diaphragm contain many abscesses. Similar lesions are also found in the deeper structures of this organ.

The *spleen* shows some perisplenitis and at the points where this occurs superficial areas of infection with the parasites or eggs are visible. On section, a moderate increase in the soft pulp is seen but no other change can be observed.

The *liver* is of normal size but adherent to the abdominal wall, diaphragm, intestine and other structures; the perihepatitis being due to the action of *Paragonimus*. On section, the organ is soft and pale and contains many small abscesses from 2 millimeters to 10 millimeters in diameter. These abscesses closely resemble the multiple amœbic ones which are so often seen, but in others the color and contents more closely resemble the *Paragonimus* cysts. Microscopically, both amœbæ and *paragonimus* eggs are found in some of these abscesses. The gall bladder and ducts are large and distended with normal-appearing bile; no parasites or eggs found.

The *kidneys* are surrounded by many adhesions containing very many abscesses of the *Paragonimus* type. Some of the lesions are subcapsular and others are in the pelves of the kidneys and in the ureters. Both organs are somewhat increased in size, soft and œdematous.

The *bladder* wall contains numerous cysts and the prostate gland is also involved.

The *pancreas* shows a few cysts near its head, these being a direct infection from the adherent structures.

The *appendix* is bound down in a mass of adhesions and abscesses and its wall is involved in the parasitic processes. The abdominal lymphatics are mostly enlarged and soft, many containing characteristic abscesses with parasites and eggs.

The *omentum* is plastered to all the abdominal structures; it is thickened, œdematous, and studded with the typical bluish abscesses.

The *stomach* is not involved excepting at points of adhesion on its outer surface. The walls are free from abscesses, and nothing abnormal is noted on the mucous surface.

The *small intestine*, from near its upper end to the cæcum, is severely infected. The *Paragonimus* lesions involve all of its coats, and are most severe in the ileum and lower jejunum. The mucous surface shows typical cysts of various sizes, some standing out well into the lumen of the bowel; ulcers resulting from broken down lesions are also present. The Peyer's patches are slighty swollen but appear to be free from the parasitic infection.

The *large bowel* in general presents a very similar picture to the small intestine, but in addition there is quite extensive amœbic ulceration extending from the cæcum to the rectum.

The *cerebro-spinal canal* was not opened.

Case No. 2 (31508).—Paragonimiasis, acute general infection, with death after eight days in the hospital; autopsy.

Nothing is known of the previous history of this patient beyond his statement that he had never been ill before. He was sick in hospital for about eight days before death with high fever, cough, and rapid, shallow respiration, abdominal pains and severe diarrhœa; he constantly complained of headache. The abdomen was moderately distended and exquisitely tender in the region of the gall bladder and over the descending colon. There was considerable difficulty in urination. Expectoration was abundant, and sometimes tinged with blood, but upon microscopic examination no tubercle bacilli or eggs were found in the sputum.

AUTOPSY four hours after death: The general nutrition is very good and slight jaundice of the conjunctivæ is visible. A macular skin eruption is noted over the lower extremities, abdomen, and flexor surfaces of arms. The muscles are soft and exceedingly pale. The pectoralis major muscles are very coarse, pale, and contain a number of small, dark spots about 1 to 3 millimeters in diameter. From one of these spots an adult *P. westermanii* was extracted. The *lymphatics* in the axilla are moderately enlarged and some of them congested.

The *left pleural cavity* contains about 750 cubic centimeters of a yellow, flocculent fluid with long, tenacious shreds of tissue. Both the visceral and parietal pleuræ are studded with raised, soft, dark-bluish colored, cystic abscess-like bodies which, on section, contain a reddish-brown granular matter. Scattered through the left lung are a number of areas of bronchopneumonia; on section most of these show one of the above-described cysts, surrounded by pneumonic areas, but in other instances the areas resemble those of ordinary bronchopneumonia. On the *right side* the general condition is similar to that on the left, but the lesions are much more extensive the entire lung being so completely filled with these pneumonic areas that it appears to be almost solid. There is marked bronchitis. Ova, but no parasites are found free in the bronchial secretion.

The *pericardial cavity* contains a small amount of normal-appearing fluid and both the visceral and parietal serous surfaces show bodies similar to those found in the pleural cavities. Over the surface of the heart itself (Pl. I) are many little, raised, brownish, granular bodies varying from 1 to 3 or 4 millimeters in diameter; these are somewhat hard, surrounded by congested zones and follow closely in their distribution that of the vessels. On section, the *heart muscle* is found to be very friable and pale, but it contains none of the bodies already mentioned. Both the upper and the lower surfaces of the *diaphragm* are studded with these small, dark, cyst-like bodies. The diaphragm itself is very much thickened and contains a number of abscesses within its substance.

The entire *abdominal cavity* is almost obliterated by a mass of adhesions, and dark, bluish cystic abscesses, varying in size from 1 millimeter to 15 or 20 millimeters in diameter, are seen in great profusion. The surfaces of the *intestine* are everywhere adherent. The omentum is thickened and filled with abscesses. The organs of the pelvic cavity are so bound by adhesions and contain so many abscesses that they can only be removed with a knife.

The *spleen* is enlarged, soft, and studded both on the surface and internally with small cystic abscesses similar to those found in the other organs. The pulp is exceedingly soft, dark-red in color, and there are numerous small areas of intense congestion corresponding to the Malpighian bodies.

The *pancreas* contains a number of the abscesses and is hæmorrhagic for a distance of about 5 centimeters from its splenic end.

The *liver* is enlarged, firmly adherent to all surrounding structures, very soft, friable and studded with little, grayish, hard areas, particularly along the connective tissue bands. A number of abscesses, mostly subcapsular, are found in this organ.

The *gall bladder* contains about 15 cubic centimeters of almost colorless bile and the ducts are open. Its wall is much thickened, firmly bound down by adhesion and surrounded and impregnated with abscesses. The *scrotum, epididymis* and left testicle contain a number of these small abscesses and the prostate gland is similarly involved.

The wall of the *large intestine* is very much thickened and is surrounded and infiltrated with abscesses. The *mucous* surface is free from ulceration, but shows a number of the previously described cystic abscesses just under its surface. This condition is most marked low down in the rectum and around the opening into the appendix. The appendix itself is involved in its walls and also externally, but not on its mucous surface. The entire *small intestine* shows the condition found in the large one, excepting that the changes are much more extensive and in several places in the ileum the abscesses in the wall have ruptured on the mucosa, leaving ulcers with ragged margins. The fibrous adhesive bands associated with broken-down cysts and abscesses in the mucosa of the bowel are very extensive and in some places they apparently almost obliterate the lumen of the bowel. The *stomach* is surrounded by a number of abscesses, but both its internal surface and wall are apparently free from the infection. The abdominal wall below the liver, on the right side, contains a knotty mass of abscesses several centimeters in diameter and extending well down behind the kidney.

Both *kidneys* are bound to a mass of proliferated tissue, abscesses and adhesions, and both of these organs contain a number of abscesses like the ones which have been described and which are most numerous just under the capsule.

The muscles of the calves of both legs and those of the thigh show abscesses which are from 3 to 5 millimeters in diameter.

The *cerebro-spinal canal* was not opened.

A *microscopic examination* shows parasites and eggs in the contents of many of the abscesses, but not in all. The former are found in the heart, lung, kidney, intestine, spleen and abdominal wall. Living parasites were extracted from the pectoralis major muscle and some were found free in the abdominal cavity; these were probably liberated by the rupture of some of the cysts during autopsy.

Case No. 3 (32625).—Paragonimiasis, extensive infection with P. westermanii; amœbiasis of the colon; death; autopsy.

The patient a Filipino, 50 years of age, has the following hospital record: Admitted to hospital October 21, 1905, with a diagnosis of acute beriberi; on October 23 he was transferred to the beriberi hospital; on February 23, 1906, he returned to the prison hospital with a diagnosis of chronic diarrhœa. From February 17 to 28 his temperature was 36°.5 to 36°.8, pulse 99 to 100, stools 2 to 11 in twenty-four hours. No cough was noted. From March 1 to 31 his temperature was 36°.5 to 37°.3, pulse 98 to 101, stools 6 to 13 in twenty-four hours. The diarrhœa was very persistent and not to be controlled by astringents or enemas. From April 1 to 30 his temperature was 36°.8 to 37°.2, pulse 98 to 100; the severe diarrhœa continued during the month and microscopic examination of the fæces, on April 9, showed amœbæ and red blood corpuscles. From May 1 to 31 the temperature was 36°.4 to 39°. The diarrhœa continued and there was moderate cough, which was worse at night. A urine examination on May 3 showed nothing abnormal and a sputum examination on May 29 was negative for tubercle bacilli and for parasites or eggs. From June 1 to 30 the temperature was 36° to 39°, the lowest point usually being during the early morning hours, but on a few occasions this relation was reversed. The cough became much worse during this month and on June 6 there was a hæmorrhage of about 100 cubic centimeters from the lungs. Examinations of the blood from this hæmorrhage and of the sputum between June 21 and 29 were negative for tubercle bacilli and for parasites and eggs. On June 29 the stool still contained amœbæ and considerable blood and mucus was also noted therein. From July 1 to 4 the temperature was 36° to 37°.2. Hæmoptysis of about 500 cubic centimeters occurred again on July 3, and an examination of this specimen was again negative. Following this last hæmorrhage the patient sank slowly and he died on the morning of July 4 with a clinical diagnosis of amœbiasis and sprue.

AUTOPSY twelve hours after death: The body is extremely emaciated, the skin normal, the subcutaneous fat very scanty, the muscles wasted and very pale and friable. The superficial lymphatics are all enlarged. One, in the left groin, and two in the right axilla are partially broken down and contain eggs of *P. westermanii.* A small, subcutaneous abscess at the back of the neck and another in the left mammary region are noted. Both contain fluke eggs. Upon opening the chest a number of dark, slate-colored, broken-down glands are found on the under surface of the sternum; a similar condition extends throughout over the parietal pleuræ on both sides. Both *pleural cavities* are obliterated by old adhesions, and all tissues, including the lung, pleural and extra pleural structures contain abscesses from 5 millimeters to 2 or 3 centimeters in diameter. Both lungs contain numerous congested areas which on section resemble bronchopneumonia. In most of these areas numerous parasites and ova are to be found. A number of the bronchial glands are broken down and have the general appearance of the other infected tissues.

The *diaphragm* is thickened, adherent to all adjacent structures and contains numerous abscesses of various sizes.

The *pericardial cavity* contains a small amount of serous fluid and numerous adhesions are present. Both the parietal and visceral serous surfaces are covered with small, raised, dark-bluish or brownish congested spots which on section are shown to contain ova; furthermore, there are at least two adult trematodes just beneath the visceral pericardium, in the region of the right auricle. The heart muscle is very brown, but no ova are found in its substance.

The *abdominal cavity* contains large numbers of adhesions between the omentum and the other structures. In several places large areas of the omentum are puckered and brought to a central point of adhesion in the abdominal wall, and at these places parasites or eggs are invariably found. The omentum in general is thickened and contains numerous abscesses similar to those in other parts of the body.

The *spleen* is enlarged and adherent to the diaphragm and abdominal wall. It contains a number of superficial abscesses, which show both parasites and ova. On section it is rather firm, bloodless and without increase of pulp. No abscesses or parasites are found in the deeper portions of the organ.

The *liver* is of normal size, adherent to the diaphragm, abdominal wall and intestine and contains numerous subcapsular abscesses. The organ is rather firm and on section shows some increase in the interstitial tissue, from which ova are to be isolated, but no abcsess exists within the parenchyma of this viscus.

The *gall bladder* is large; it contains a considerable quantity of light-colored, fluid bile, no parasites or eggs are seen either in the gall bladder or ducts. Nothing abnormal is found in the portal vessels.

Both *kidneys* are of normal size. They are very pale and soft, with nonadherent capsules. The left kidney contains two subcapsular abscesses and one is seen in the pelvis. The adrenals appear to be normal.

The left *psoas muscle* contains an abscess with about 100 cubic centimeters of matter having the appearance of anchovy sauce, in which hundreds of parasites and eggs are encountered. There are a number of smaller abscesses in the posterior abdominal wall, on both the right and left sides.

The *prostate gland* and *epididymis* contain a few abscesses.

The *bladder* appears to be normal.

The *entire large intestine* is thickened, of a dark-slate color, and it shows a number of hard nodules beneath its peritoneal surface. On section, numerous ulcerations extending from the rectum to the cæcum are found. These ulcers vary in size from 2 millimeters to 10 millimeters. They bear no relation to the mesenteric border or to any other definite anatomic structure. Their margins are raised, appear as if they were punched out, are rather hard, granular and sometimes intensely congested; in other instances they are without congestion. The floor of these ulcers is either in the submucosa or it extends down to the muscular layer. They vary much in size, contain a dark-colored, thickened matter, together with numerous ova and in one instance a parasite.

The lower portions of the *small intestine* and *appendix* are similar to that of the large, but the upper two-thirds of the small bowel is apparently free from parasitic infection.

The *stomach* and *pancreas* appear to be normal.

A most striking general picture of this autopsy, as well as in the two preceding ones, is found in the peculiar, dull-slate color of all the tissues involved in the infection. This is particularly noticeable in the omentum, intestinal wall, and in the other tissues of the abdomen.

The abscesses referred to are of a peculiar appearance and are characteristic. They vary very much in size, but the general appearance in every instance is the same. There is a rather definite limiting cystic wall, of a dull-blue slate color, and on section a dark material like anchovy sauce is found. In most instances these abscesses contain either parasites or ova, or both.

Case No. 4 (37995).—Jacksonian epilepsy, paragonimiasis of the brain, lungs and abdominal organs; death, autopsy.

The patient, a male Filipino, 30 years of age, was admitted to the hospital with an epileptic seizure and lived about twenty-four hours, during which time the attacks were repeated two or three times. Nothing is known of the previous history of this patient except that he was in the hospital on two occasions during the present year suffering from acute coryza. During his last illness there was no fever, and the epileptic attacks were typical in every way of epilepsy major.

AUTOPSY four hours after death: Body of a well-developed, well-nourished Filipino man. Skin and mucous membranes appear normal. Two small, bleeding surfaces on the tongue, evidently from injury during his epileptic seizures. There is some œdema of the lower eyelids and a very marked, chronic conjunctivitis. The mucous membrane of the nose is also hypertrophied and œdematous. The subcutaneous tissues contain a moderate amount of fat and are normal in appearance; the subcutaneous lymphatics are not enlarged, except in the right axilla, where there is moderate hypertrophy without any evidence of an acute process.

The *muscular tissues* are rather coarse and quite pale in color. Both *pleural cavities* contain a small amount of cloudy, serous fluid and a number of chronic adhesions, these adhesions being diffuse over the visceral surface and collected in points of attachment on the parietal surface. At these points of attachment, along the ribs, are found a number of slate-colored, cystic abscesses which, on section, are typical of those already described for paragonimiasis. Both *lungs* are rather voluminous and there is a marked bronchitis of all of the larger and medium sized tubes. In the left lung there are several areas of bronchial pneumonia due to *P. westermanii*. No parasitic areas are found in the right lung.

The *visceral* and *parietal* layers of the *pericardium* are adherent in several places and, on separation of these adhesions, there are a number of raised, brownish, granular patches which contain eggs of the parasite in question. The heart muscle itself is firm and normal in color and no lesions of the endocardium are found.

The *abdominal cavity* is free from adhesions, except around the duodenum, where there is a mass of old adhesions surrounding a post-peritoneal abscess which leads well above and behind the left kidney; this contains about 50 cubic centimeters of material resembling anchovy sauce and a number of adult parasites and eggs. No direct connection between this abscess and any of the organs can be established.

The *spleen* is of normal size and consistency and no evidence of disease in it was discovered on section.

The *liver* is slightly cirrhotic and the gall bladder and bile ducts are moderately distended with fluid, which microscopically is free from any abnormal elements. No abnormal changes were found in the kidneys, bladder, or other of the pelvic or abdominal organs.

The *intestine* unfortunately was not opened.

The membranes of the *brain* are thickened and adherent in many places; bound in these adhesive masses are cystic parasitic abscesses similar to those of the pleural cavity. The choroid plexus is coarse and granular, slightly bluish, and a few eggs are found in its substance. The brain substance itself appears to be normal, with the exception of moderate congestion near the cortical surface. No distinct abscess cavity was found in this organ.

Case No. 5.—Pulmonary tuberculosis; pulmonary pleuritic, diaphragmatic, omental, intestinal and lymphatic paragonimiasis; death; autopsy.

Practically nothing is known of the clinical history of this case; the patient continued to perform his duties to within about twenty-four hours of death, at which time he first came under observation; no microscopic examinations were made during life. When admitted to the hospital he was very weak, moderately emaciated, was coughing considerably and complained of pains in his left chest and abdomen. There was an active diarrhœa and a slight elevation in temperature.

AUTOPSY eight hours after death: Pulmonary tuberculosis and a rather extensive infection with *P. westermanii* are found. The tuberculosis involves the bronchial glands and the upper lobes of both lungs. Some of the lesions consist of caseous areas, from 2 to 5 millimeters in diameter, and there are also several tuberculous cavities of about equal size, and one 10 millimeters in diameter is found in the apex of the left lung. Very few miliary tubercles are encountered.

The *Paragonimus* lesions of the *lungs* are very much scattered, as are the tuberculous ones, and they so closely resemble the cavities of tuberculosis that had it not been for the more extensive distribution of the *Paragonimus* cysts in other organs, the double infection in the lungs would probably not have been discovered. Many of the tuberculous lesions show a bluish color, similar to that constantly observed in those due to Paragonimus infection and it is worthy of note that one cavity contains both tubercle bacilli and an adult *P. westermanii.*

The *Paragonimus* lesions in the *pleuræ, diaphragm* and *abdominal organs* are similar to those described in the previous cases.

Case No. 6.—Paragonimiasis of intestine, omentum, pancreas, diaphragm, abdominal lymphatics, and left pleura; chronic beriberi; death; autopsy.

The patient, a native male about 40 years of age, was admitted to hospital for chronic diarrhœa, which he stated was of long standing; he also said that he had suffered from acute beriberi about eight months before the time of his admission and that he was still suffering from this disease. Death from exhaustion and intestinal hæmorrhage occurred five days after he came under observation. On admission, the patient complained of great weakness, diarrhœa, and partial paralysis, principally involving the lower extremities. There was no cough and no pain in the chest. Emaciation was very great and anæmia pronounced. There was moderate atrophy of the lower extremities and all the reflexes were absent.

AUTOPSY nine hours after death: Paragonimus lesions similar to those already described under the other cases are found in the *intestinal wall,* the *peritoneum, omentum, mesenteric lymphatics, diaphragm, small intestine* and *left pleura.* No such lesions are observed in the *lungs,* although a most careful search was made, and these organs are normal, excepting a moderate congestion and œdema. The *left pleura,* particularly on its parietal layer, is moderately infected and this condition is more marked toward the base of the chest, this infection apparently being continuous with the lesions along the diaphragm. The most advanced lesions are found in the *abdominal cavity,* and particularly in the omentum, where some of the slate-blue cysts 10 to 15 millimeters in diameter are observed.

Lesions other than the specific and definite cysts due to *Paragonimus* infection are as follows: Moderate, fatty degeneration and passive congestion of the liver; slight increase in the size of the spleen with some fibrosis and perisplenitis; moderate cirrhosis of the kidneys and old, healing amœbic ulcerations of the colon.

As is true of the other cases studied, parasites or eggs are found in many, but not in all the cysts.

Case No. 7.—Intestinal, omental, and lymphatic paragonimiasis, acute dysentery and chronic parenchymatous nephritis; death; autopsy.

Practically nothing is known of the clinical history of this patient. The most interesting feature of the case is the complete absence of lung or other thoracic lesion, in the presence of a well-defined *Paragonimus* infection of some of the abdominal organs. There are two typical lesions due to *P. westermanii* on the under surface of the diaphragm, on the left side.

Case No. 8.—Paragonimiasis of the left lung, pleura and superior surface of diaphragm; acute generalized tuberculosis; death; autopsy.

The interesting features of this case are as follows: The parasitic lesions are confined to the thoracic cavity; they are associated with tuberculosis and the similarity between some of the tuberculous and the *Paragonimus* lesions is striking. The bluish color of the parasitic abscesses is less marked than in the other cases and a similar appearance of the abscesses of tubercular origin is more decided than it is usually. Tubercle bacilli and the eggs of *P. westermanii* were found together in some of the lesions.

Cases Nos. 9 to 17.—These are all cases which have only been observed clinically, in which the diagnosis has been made by the finding of the eggs, either in the sputum or stool. These cases will not be reported in detail but the more or less careful clinical findings will be embodied in their appropriate places in this paper.

VI. ETIOLOGY.

A. CONTRIBUTING CAUSES.

The contributing causes which bring about infection with this organism, of course, can not completely be elucidated until the life cycle of the parasite has been fully worked out. However, a number of points deserve consideration.

(*a*) GEOGRAPHICAL DISTRIBUTION.—In the opinion of some of the best zoölogists, not all of the parasites which have been reported as *Paragonimus* are of the species *westermanii,* but until such time as this confusion is made more clear, it seems advisable to consider the geographical distribution of this disease as including all points from which the parasites have been reported either for man or other animals. Considered in this broad sense, the geographical distribution would include practically all of Japan, points in Korea, Formosa, China, Holland (Amsterdam), Germany (Hamburg), Sumatra and the United States, and to this list the Philippine Islands must now be added. It is almost certain that the increased interest which the importance of this subject demands will result in the finding of the infection in many places where it has hitherto been unsuspected.

(*b*) AGE, SEX, RACE, OCCUPATION, CLIMATE, AND PERSONAL HABITS.—*Age* is probably not a factor in the etiology of the disease, excepting to the extent that, as in many other infections, the exposure is greater and the infection correspondingly more frequent during the more active years of life. Stiles from the statistics up to 1900, compiled 59 cases, 45 of which were between the ages of 11 and 30 years. Four cases were in children under 10 years of age and one in an individual over 50 years old. My 17 patients ranged from 20 to 62 years of age. Eleven of them were between 25 and 35.

Sex.—The disease is much more frequent in males than in females. This has been noted by several Japanese observers and in a recent conversation with me, Professor Tokishige again emphasized this fact, at least in so far as Japan is concerned. Stiles and Hassall's statistics show 58 males out of 66 cases. My cases have all been in males, but it must be understood that in compiling this series my opportunities for observation have not been such as to allow me to judge in regard to females.

Occupation.—The majority of writers attribute very little importance to occupation as a factor in the etiology of the disease. Many of the reported cases have been among those whose vocation brought them in contact with soil and water. My cases have all been among people of the lower classes, where many of the different occupations offer similar types of exposure and where the vocation is often changed from day to day. Of 9 patients in my series where the occupation was given, 6 were fishermen, 2 were cooks and 1 a laborer. So far as I know, fishermen have not been considered especially susceptible in Japan.

Climate.—Katsurada states that the disease is more common in the mountainous region of Japan.

Physical condition and personal habits.—Some writers have considered cold and other infections to be predisposing factors, while others state that healthy persons are equally susceptible.

An examination of my cases does not indicate any especial susceptibility due to the personal habits or to the physical condition of the patient. As may be noticed from the protocols, other diseases, which probably antedated the fluke infection, were present in some instances, while in others the parasitic disease was apparently the only contributing cause of death. The association of tuberculosis, amœbiasis and other diseases with paragonimiasis will be discussed in another part of this paper.

B. SPECIFIC ETIOLOGY.

TREMATODES IN GENERAL.—The zoölogical position of parasitic flukes is shown in Table No. 1, and the principal diagnostic points of the parasites and ova are given in Tables Nos. 2 and 3. In addition to this, however, it seems advisable to enter somewhat more into the zoölogical details, because of the very great importance of the infections to which trematodes may give rise.

There are two classes of the *Platyhelminthes* or flat worms of interest to medical men. These are the *Cestoda,* which have no digestive tube, and the *Trematoda,* which have a more or less well-developed digestive apparatus without an anus. The order *Malacocotylea* of the class *Trematoda* contains 4 families and 10 genera or collective groups, with 15 species which interest us as medical men. There are certain more or less general characteristics of the members of the order which here may briefly be noticed, because they tend to simplify the study of the species which directly concerns us.

Nearly all parasitic flukes are hermaphroditic, with two important exceptions, namely, the two species of the genus *Schistosoma* (*S. hematobium* Bilharz and *S. japonicum* Katsurada), in both of which male and female adult parasites develop. They are all rather small, nonsegmented worms of various shapes, but for the greater part they are of an indefinite oval form. There are one or more suckers and in a few instances one of these is armed with hooklets. The mouth or oral sucker also performs the function of the anus. The *cuticle* in most species is in part covered with scales or spines, but these are entirely absent in others. The *muscular* system is rather poorly developed, it consists of a series of fibers just beneath the cuticle and what is known as the parenchymatous muscle, which sends branches throughout the body. A *nervous* system, also poorly developed, consists chiefly of two or three ganglionic masses near the head, with their corresponding trunks and branches to various portions of the body. The *digestive* tract consists of a mouth (oral sucker), pharynx, and œsophagus, which divides into two intestinal cæca, which in turn, in a more or less winding manner, reach to the posterior portion of the parasite and terminate as blind sacs. In the genus *Schistosoma* there are one or more unions and separations of these intestinal cæca before they end in the posterior portion of the parasite The *excretory* system is primitive, but fairly well developed. It begins in excretory cells located in various parts of the body which lead by channels with the usual anastomoses, to an opening in the posterior portion of the parasite, called the excretory pore. The *reproductive* system is well developed and shows considerable variation in different species. In the main, the prominent features are about as follows: The two testicles are usually situated in the posterior portion of the worm, and are of various shapes. Each one is connected by a seminal duct with the *vas deferns*, which in turn passes forward and ends in the cirrus pouch which is on the ventral surface and closely connected with the genital pore. This pouch also contains the cirrus or penis. From the vulvar opening the vaginal tube is continuous with a well-developed uterus which usually has many folds and terminates in the shell gland at the cotype. It is indirectly continuous through the cotype with other tubes which also enter the latter as follows: The oviduct which connects directly with the ovary, the vitelline duct which is formed by tubes connecting with the yolk glands or vitelline follicles and Laurer's canal, which is a small canal of unknown function opening on the back of the worm and often distended at its inner end into a *receptaculum seminis*. Ova pass out through the vaginal outlet and at the time of oviposition may or may not contain a ciliated embryo. In either case, under proper environment, a ciliated miracidium escapes from the egg and after a time enters an intermediate host. The parasite is again taken into an animal host from some one of these unknown intermediate ones and the adult parasite once more developes.

The entire life cycle of any of these flukes with the exception of that of *F. hepatica* Linn. is not known, but it is probable that the intermediate hosts will be found among the same class of mollusks as the ones which harbor *F. hepatica.*

Oversized
Foldout

TABLE No. 1.—*Trematodes infecting man, showing the zoölogical position of flukes.*

(Order.)	(Family.)	(Genus, or collective group.)	(Species.)
Malacocotylea	Monostomidae	Monostomulum	*M. lentis* Gescheidt.
	Fasciolidae	Agamodistomum	*A. ophthalmobium* Diesing
		Opisthorchis	*O. felineus* Rivolta.
			O. noverca Braun.
			O. sinensis Cobbold.
		Fasciolopsis	*F. buskii* Lankester.
			F. rathouisi Poirier.
		Heterophyes	*H. heterophyes* Siebold.
		Paragonimus	*P. westermanii* Kerbert.
		Fasciola	*F. hepatica* Linn.
			F. gigantica Cobbold.
		Dicrocoelium	*D. lanceolatum* Rudolphi.
	Paramphistomidae	Gastrodiscus	*G. hominis* L and McC.
	Schistosomidae	Schistosoma	*S. haematobium* Bilharz.
			S. japonicum Katsurada.

P. WESTERMANII KERB.—*P. westermanii* belongs to the family *Fasciolidae* and is the type species of the genus *Paragonimus.* It was discovered by Kerbert in 1878.

(*a*) *Synonyms.*—The synonyms for this parasite as given by Stiles are in the foot note.[1]

(*b*) *General description and specific diagnosis of the parasites.—Size:* There are considerable variations in the measurements of the this worm as they are given by various writers. These are shown in Table No. 2. The personal equation must be considerable in trying to measure the living organism but, even after eliminating this and as much as possible all other sources of error, the differences in measurements are very large and they are often noticeable in mature specimens from different locations in the same host. The limits of variation are probably included in the following measurements: 0.008–0.020 × 0.004–0.009 × 0.002–0.006. On one occassion several parasites found in an abscess in the abdominal cavity were quite large, averaging 0.015–0.018 in length, whereas those taken from the lung in the same case were only 0.010–0.015. The worms contract under the influence of all preservatives, and measurements made with specimens which have been subjected to this influence give still more uncertain results.

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[1] (*a*) SYNONYMS.—*Distoma westermanii* Kerbert, 1878; *Distoma ringeri* Cobbold, 1880; *Gregarina pulmonum* Baelz, 1880; *Gregarina fusca* Baelz, 1880; *Distomum westermani* Kerbert, 1881; *Distoma pulmonis* Kiyona, Suga, and Yamagate (1881); *Distomum pulmonis* Kiyona, Suga, and Yamagate (1881); *Distoma pulmonale* Baelz, 1883; *Distoma pulmonar* La Clinica de Malaga, 1883; *Distoma pulmonum* (Baelz) Tomono Hidekata, 1883; "*Distoma hepaticum* Linn." of Miura, 1889; *Distomum ringeri* (Cobbold, 1880) von Linstow, 1889; *Distomum westermanni* Leuckart, 1889; *Distomum pulmonale* (Baelz) Leuckart, 1889; *Distomum cerebrale* Yamagiwa, 1890; *Distoma ringers* Rev. Sci., 1890; "*Mesogonimus westermanni* (Kerbert, 1878)" Railliet, 1890; *Mesogonimus pulmonalis* (Baelz, 1883) Railliet, 1890; *Mesogonimus ringeri* (Cobbold, 1880) Railliet, 1890; *Distoma westermanni* (Kerbert, 1881) Weber, 1891; "*Distoma westermanni* (Leuckart) Blanchard, 1891; "*Mesogonimus pulmonale* (Baelz, 1878)" Stossich, 1892; "*Distoma* (*Mesogonimus*) *westermani* Kerbert, 1878," of Stiles, 1894; *Distomum* sp. of Kellicott, 1894; *Distomi ringeri* (Cobbold, 1880) Simon, 1897; *Paragonimus westermanii* (Kerbert, 1878) Stiles & Hassall, 1900.

Color: The color, when the trematode is first removed from the animal host, varies from a dark reddish-brown to a light slate. After it has been exposed to the air for a short time, the surface takes on a dull-grayish appearance, similar to that seen in the preserved specimens.

Shape: When the parasite is alive its shape is somewhat variable, but on the whole it is fairly constant; it is oval, the more acute pole being the caudal extremity. The ventral surface is more flattened than the dorsal one and at times it is even concave, except at the extreme cephalic end, where the more spherical shape is maintained. Specimens are always altered while in preparation; this is probably due to the more rapid absorption of the chemicals by certain portions of the body and the consequent uneven contraction.

Motility: There does not appear to be much in the literature concerning the motility of these worms. The parasites do not appear to have more than a slight power of active locomotion when they are removed from the host, but motions of different portions of the body may be seen. The most interesting of these gives the trematode the ability to protrude and retract its head by stretching or contracting that part of the body cephalad of the acetabulum, and again it has the power of altering the plane of the oral sucker in a like manner, so that at times the latter may appear to be directly terminal and again almost completely subterminal. These two motions may be observed in a much more striking degree in members of the genus *Opisthorchis.*

Cuticle: A rather thick, dense skin envelopes the entire parasite, but when it is carefully studied in serial sections cut longitudinally and transversely (see Plates V, VI, VII, and VIII) it is seen that this cuticle is not of equal thickness throughout, it being heavier on the dorsal and lateral surfaces than it is on the ventral one. Even in some places on the dorsum it is thicker than it is in others. At the cephalic end, the structure of the cuticle is more closely interwoven with that of the deeper tissues.

Spines: Almost the entire body of this worm is covered with scale-like spines, which are of different sizes in different locations, and in certain places, such as the ventral surface from the acetabulum to the oral sucker, they are almost entirely absent. The spines in this situation, wherever they occur, are smaller than the others; they gradually increase in size on the lateral borders and they reach a maximum on the dorsum. This seems to be true whatever transverse plane of the body is studied, but it is particularly marked about the region of the acetabulum. Again, in longitudinal sections, it is observed that the spines at the extreme cephalic extremity are smaller and that they become larger toward the equatorial portion and again slightly smaller at the caudal extremity. In places, such as on the dorsum, these spines seem to extend above the surface more than they do in other situations, whereas along the caudal portion of the ventral surface their projection above the surface is hardly noticeable in sections. The varying direction of these spines as they are seen in sections from different worms would suggest that, during life, the animals may have the power of moving them and, perhaps, of using them in locomotion or in holding themselves in position for other purposes. This hypothesis of motion is further born out by the unique arrangement and setting of the spines in the skin. They rise from the inner surface of this organ, penetrate it at fairly constant and parallel angles, and are very closely held in place. Alternate layers arise from a slightly more superficial layer of the skin, but these pass out, as do those from the deeper tissues.

Oral sucker: This is spherical or slightly oval in shape, the largest diameter always being ventro-dorsal, it is terminal to subterminal, and in the living parasite it may change from one to the other of these positions. Measurements have only been possible in preserved specimens, and here accurate work with a Zeiss photomicrographic apparatus gives 0.86 millimeter as an average of 10 specimens, the smallest being 0.63 and the largest 0.97 millimeter. The sucker is composed of outer and inner, rather definite, membranes which are connected by radiating fibers interspersed with small, granular cells. The outer membrane apparently connects directly with the parenchymatous muscle fibers of the parasite. The lateral lips of the sucker are heavier than the ventral and dorsal ones.

Ventral acetabulum: This is slightly larger than the oral sucker. The average measurements in the parasites used in measuring the oral sucker gave 0.92 millimeter, the smallest being 0.76 and the largest 1.31. It is slightly oval or spherical in shape, and in structure it closely resembles the oral sucker. It is situated in the cephalic half of the body, on the ventral surface. At times its surface seems to be parallel with the ventral surface of the parasite and again it appears to form quite an angle with the latter. The distance of the acetabulum from the oral sucker is a changing one during the life of the parasite; this is due to contractions and protrusions of the cephalic portion of the worm.

Pharynx: This is a constricted portion of the oro-œsophageal tube and it does not differ in structure from the œsophagus.

Œsophagus: This organ is short and finally divides into the two intestinal cæca. Together with the pharynx, it arches somewhat dorsally from the oral sucker to its bifurcation; in structure it resembles the oral sucker.

Intestinal cœca: These, two in number, rise from the bifurcation of the œsophagus and run out in a zig-zag course on either side, very nearly to the caudal end of the parasite. Sections through these organs show what appears to be a primitive mucous membrane consisting of a basement membrane with superimposed cells

Genital pore: This is small and often indistinct. In 3 out of 5 specimens it was to the left of the median line, close to the ventral acetabulum.

Male organs: These are not very well developed; the testicles lie one on each side of the median line, the one slightly more caudad than the other, and both just posterior to the uterus, however; the latter organ sometimes so overlaps one of the testicles as to render its detection difficult. The cirrus and cirrus pouch are absent.

Female organs: The rather short, compact, closely coiled uterus may lie on either side of the median line just caudad of the ventral acetabulum. The ovary is distinct, branched, and usually almost opposite and slightly posterior to the uterus.

Excretory pore: This is prominent and situated at the caudal extremity.

Vitellaria: These are numerous and well developed, covering almost the entire parasite. Laurer's canal is present.

TABLE No. 3.—*Showing the principal diagnostic points of fluke eggs.*

	Size.	Shape.	Color.	Operculum.
M. lentis Gescheidt	--------	--------	--------	
A. ophthalmobium Diesing.	--------	--------	--------	
O. felineus Rivolta	Styles, 26–30 × 11-15	Oval	(?)	Well defined on the more acute pole.
O. noverca Braun	34 × 19–21	do	--------	(?)
O. sinensis Cobbold	27–30 × 15–17	do	Dark brown	Sharply defined.
F. buskii Lankester	120–130 × 77–80	--------	--------	
F. rathhouisi Poirier	150 × 80	Ovoid	--------	
H. heterophyes Siebold.	20–30 × 15–17	Oval	Light brown	(?)
P. westermanii Kerbert.	Leuckart, 80–100 × 56; Ward, 96-118 × 48–53; Stiles and Hassall, 68–96 × 48–60.	do	Yellow shell	Present.
F. hepatica Linn	130–145 × 70–90	do	--------	
F. gigantica Cobbold	150–190 × 75–90	--------	--------	
D. lanceolatum Rudolphi.	38–45 × 22–30	--------	--------	
G. hominis Lewis and McConnell.	150 × 72	Oval	--------	Do.
S. haematobium Bilharz.	135–160 × 55–66	do	--------	Not present; usually terminal or subterminal spine.
S. japonicum Katsurada.	58–90 × 30–72.5; Woolley, 62.4–72.8 × 43.6–48.	do	Yellow brown.	No spine or operculum.

TABLE No. 3.—*Showing the principal diagnostic points of fluke eggs*—Continued.

	Miricidum.	Sporocyst.	Redia.	Cercaria.	Intermediate host.
M. lentis Gescheidt					
A. ophthalmobium Diesing.					
O. felineus Rivolta	Contains ciliated embryo at oviposition.	Not known	Not known	Not known	Unknown.
O. noverca Braun	(?) Unknown	do	do	do	Do.
O. sinensis Cobbold	Contains ciliated embryo at oviposition.	do	do	do	Do.
F. buskii Lankester		do	do	do	Do.
F. rathhouisi Poirier		do	do	do	Do.
H. heterophyes Siebold.	Thick shell; contains ciliated embryo at oviposition.	do	do	do	Do.
P. westermanii Kerbert.	Ciliated; developes after egg leaves the host.	do	do	do	Do.
F. hepatica Linn	Conical, ciliated, with one papilla, two cup-shaped eye spots and rudimentary intestine.	Found in small snails of genus *Linnae.*		Whitish and found encysted on plants.	Snails.
F. gigantica Cobbold					
D. lanceolatum Rudolphi.	Contains embryo when oviposited.				
G. hominis Lewis and McConnell.					
S. haematobium Bilharz.	Develops after oviposition.				
S. japonicum Katsurada.	Elongate, oval, ciliated on anterior portion.				

Ova: The eggs are oval in shape and vary in color from a reddish-brown to light-yellow, the color depending somewhat upon the stage of their development and the source from which they are obtained and perhaps, also, upon other conditions which are not fully understood. Again, their size varies considerably, and a glance at Table No. 3 will show the marked differences in the measurement given by some of the principal observers. I have carefully measured several specimens with the Zeiss photomicrographic apparatus with the following results:

Length, 0.0062 to 0.0098; average, 0.0074.
Breadth, 0.0047 to 0.0063; average, 0.0057.

Eggs in sections of tissues and those preserved in any other manner contract somewhat and these are not considered in the measurements given above. A well-marked operculum is probably present in all mature specimens, but in many instances this is indistinct and at times it can not be made out at all. This operculum has quite a distinctive appearance which is noticeably different from that of some of the other fluke eggs. The operculum in the ova under consideration appears to fit into an opening very much in the manner of a cork into a bottle, whereas in some of the other trematodes, particularly *Opisthorchis,* the opercula appear to be placed over openings more in the fashion of a cap.

The *ovic cell* in many specimens is quite distinct, and several *yolk cells* are then also present, but in others, even if they are taken from the same abscess, no such structure can be made out.

(*c*) *Life cycle.*—The life cycle of this worm is unknown; this fact is brought out by Table No. 3. When the eggs are oviposited they do not contain an embryo, but Manson and a few other observers have succeeded in hatching free swimming, ciliated miracidia from these eggs by placing them in water for given lengths of time. I have repeated these experiments many times, but so far have not succeeded in obtaining the miracidia. The sporocyst, redia, cercaria and intermediate host or hosts are absolutely unknown, but reasoning from analogy it is probable that the intermediate host will be found to be some form of edible snail.

(*d*) *Habitat.*—The habitat is in man (all tissues and organs) the dog, cat, hog, cow and tiger. In the Philippine Islands it has been found in man and the cat. The men under my observation were one Chinaman and two Japanese, the others being native Filipinos. Thirty-two autopsies on cats were performed and although fluke infections were present in over 60 per cent of the animals, *P. westermanii* was found but once. It has not been encountered, even after careful search in rats, dogs, or monkeys.

(*e*) *Distribution in the body.*—The parasites or eggs have been reported according to previous literature in the following places: The lung, brain, eyelids, liver, intestinal wall, omentum, diaphragm, cervical glands, in Poupart's ligament, the perineum, appendix, rectum, peritoneum, cirrhotic liver, and free in the abdominal cavity.

Looss and other careful workers doubt the diagnosis in some of these cases. However that may be, I now wish to confirm every one of the above-mentioned sites of infection and to add to them practically every other tissue and organ in the body. Particular attention may be called to the spleen, skin (ulcers), lymphatics, pancreas, heart and pericardium, the epididymis, urinary bladder, psoas and other muscles, and many other places. In fact, in some of my cases which have been described above the distribution of the parasites, eggs and lesions was sufficiently universal to justify the term of *general infection.*

(*f*) *Primary points of infection and manner of spread in the body.*—The exact mode of infection can not, of course, be determined until the life cycle of the parasite is further elucidated. Considering the facts which are available, it has generally been supposed that the infection is taken into the gastro-intestinal canal through food or drink. However, it has been difficult to reconcile this conclusion with the next, quite generally accepted, statement that the lungs are the primary seat of lesions and, indeed, are the only organs involved in many of the cases. Several observers believe that the worms reach the lungs by wandering from the œsophagus into the trachea and then through the bronchial tubes. Katsurada considered that the young worms could bore through the intestinal wall and reach the lungs by the lymphatics and Yamagiwa thought that they could enter them by penetrating directly through organs and tissues. All have agreed that the brain lesions which are often encountered are secondary to the pulmonary ones, and that the infection reached this organ through the blood vessels.

Considering our present knowledge of the subject, infection through the gastro-intestinal canal must be accepted but the possibility of infection through the sound or broken skin can not be excluded. Furthermore, from the facts brought out in this report, the lungs can not be considered as the primary seat of lesions nor even in many cases as the most important organs involved.

The observations made in Manila further convince me that the spread of the infection, when it is once established, is chiefly by the lymphatics, as was originally pointed out by Katsurada. Many facts tend to support these statements. In the first place, in at least one of my cases the lungs were not at all involved and in one instance, they were the only organs infected. All the findings are compatible with the theory of gastro-intestinal infection, spread by lymphatics. Even the lung lesions often bear out this view, for, as we shall presently see, the majority of the lesions are not of the bronchiectatic type as it is generally considered, but they bear a closer relation to the lymphatic system.

VII. PATHOLOGY.

The articles dealing with the pathology of this infection prior to 1899 have been well summarized by Stiles and Hassall; the most important ones since that time have been those of Katsurada, Scheube and of Looss. Taken together, these discussions hardly present an adequate picture of the pathology of the disease as it has been seen in Manila.

In general in my cases, the distribution and severity of the lesions have been greater than is usually recognized to be the case by other writers, and the lung lesions are not as predominant as they have been accredited with being in other localities where the disease is endemic.

A. MATERIAL.

The character and the amount of material for this report has already been extensively reviewed in Chapter V.

B. METHODS.

Museum specimens have been prepared by the Kaiserling method and a fairly complete collection of specimens deposited in the Museum of the Biological Laboratory of the Bureau of Science in Manila. Another smaller selection has been forwarded to the Army Medical Museum in Washington, and still others furnished the Naval School of Tropical Medicine in Washington and other interested persons. Tissues for histologic study were fixed in Zenker's solution and in alcohol and ultimately imbedded in paraffin.

A large variety of stains has been used, including Van Gieson's, Heidenhain's iron and other hematoxylins, Borrel's and the safranin stains. Many stains are satisfactory for the reason that fine differentiation is hardly necessary for the study in sections of the organisms or eggs. On the whole, the Van Gieson has proved the most useful stain, and it is by far the most satisfactory for the study of the cuticle and spines of the parasites. Eosin and methylene-blue give a sharp and clear picture, particularly of the eggs, although these, as well as the parasites, are easily recognized even in unstained sections.

TABLE No. 4.—*Showing pathologic summary*

	I.	II.	III.
External findings	Emaciation and anæmia, marked chronic conjunctivitis. Bluish ulcer right axilla containing ova *P. westermanii.*	General nutrition good; moderate jaundice; macular skin eruption.	Emaciation extreme. Small, subcutaneous abscesses at back of neck and left mammary region and in left groin containing ova.
Lymphatics	Necrotic in right axilla and contain ova *P. westermanii.* Superficial, all moderately enlarged. Inguinal and femoral, markedly enlarged. Abdominal enlarged, some necrotic and contain eggs of *P. westermanii.*	Axillary moderately enlarged and congested.	Superficial, all enlarged, one in left groin and two in right axilla broken down and contain ova. Bronchial glands bluish and contain abscesses.
Muscles	Soft, pale and coarse. Adult *P. westermanii* in left pectoralis major and another in cyst cavity in abdominal wall.	Soft and pale. Both pectoralis major muscles contained cystic abscesses with adult *P. westermanii.* A chain of abscesses in abdominal wall on right side extending down behind the kidney. A few small abscesses in the calf muscles of both legs.	Wasted. Large left psoas abscess containing over 50 adult parasites; other small abscesses in posterior abdominal wall on both sides.
Thoracic cavity.	Many adhesions. Bluish *P. westermanii* cyst on under surface of sternum.	A few small, bluish, cystic abscesses on under surface of sternum.	Dark, slate-colored cystic abscesses on under surface of sternum.
Pleuræ	Considerable fluid with flocculi and stringy material in both. Many adhesions, both visceral and parietal, contain many bluish *P. westermanii* cysts with eggs. Some are in chest wall outside pleura.	Both cavities contain yellow, flocculent fluid with long shreds of necrotic tissue. Both visceral and parietal pleuræ and even external to the pleuræ are very numerous, bluish cystic abscesses containing ova or parasites.	Extensive obliterative pleuritis. All tissues and even extra pleural structures infiltrated with typical abscesses.
Lungs	Moderate bronchitis. No bronchiectatic cavities and no eggs or parasites free in bronchi. Numerous areas of bronchopneumonia, largest near surface. Many of these areas contain cysts with *P. westermanii,* and others contain ova. Many bluish cysts with eggs or parasites not in the pneumonia areas.	Moderate bronchitis with ova but no parasites free in the tubes. Both organs contain many areas of broncho-peumonia, the largest one being superficial. Some of these areas appear as those of ordinary pneumonia while others surround cystic abscesses. There are also numerous bluish cystic abscesses, more numerous subpleural, which are not surrounded by pneumonic areas.	Extensively involved; areas of bronchopneumonia and abscess formation as in Cases I and II.
Pericardium	Small amount of cloudy fluid but no eggs. Serous surfaces dull and adherent over right auricle. Eggs and parasites in adhesions in brownish patches.	Normal amount of clear fluid, a few adhesions between the epi- and pericardium. Both membranes show small, brownish areas containing ova. Several bluish cystic abscesses on outer surface of parietal membrane.	Extensive obliterative pericarditis. Both parietal and visceral surfaces covered with dark, brownish, raised areas containing ova. Two adult parasites under visceral pericardium. (See Pl I.)
Heart	Muscle brown and coarse; *Paragonimus* lesions extend a short distance from pericardium.	Surface shows many little brownish raised areas which contain ova and follow the vessels in distribution. Muscle pale and friable.	Muscle very brown. For surface see above and Plate I.

in eight fatal cases of paragonimus infection.

IV.	V.	VI.	VII.	VIII.
Well developed and nourished. Some œdema of eyelids and marked chronic conjunctivitis. Mucosa of nose hypertrophied.	Moderate emaciation.	Extreme emaciation and anæmia.	----------	----------
Superficial, normal except in right axilla where there was moderate congestion.	Not involved except in the abdominal cavity.	Superficial, normal; mesenteric and other abdominal enlarged and may contain *Paragonimus* abscesses and eggs.	Moderate *Paragonimus* infection of the abdominal lymphatics.	----------
Appear normal ----------	Pale and soft ---	Pale and wasted.	----------	----------
----------	----------	----------	Absolutely free from *Paragonimus* lesions.	----------
Moderate obliterative; pleuritis. Moderate number of the *Paragonimus* abscesses in left side and a few in the right	Very extensive *Paragonimus* infection similar to Case I.	Right normal, left contains a few *Paragonimus* abscesses and adhesions.	Appear normal.	*Paragonimus* lesions of left side.
Marked bronchitis of the larger tubes but no eggs or parasites. A few pneumonic areas in the left lung and a few bluish abscesses. The right lung apparently free from the fluke infection except in areas immediately beneath the pleura.	Extensive double infection of tuberculosis and *Paragonimus*. One abscess contains both tubercle bacilli and adult *Paragonimus*.	Moderate congestion but no *Paragonimus* lesions or eggs found after a most careful search.	Appear normal.	*Paragonimus* lesions of left. Tuberculosis both lungs.
Adhesions in several places which, when separated, have brownish areas containing ova.	Not involved ---	Appears normal.	Appears normal.	----------
Appears normal ----------	Not involved ---	Appears normal.	Appears normal.	----------

TABLE No. 4.—*Showing pathologic summary in*

	I.	II.	III.
Abdominal cavity.	All tissues matted together and numerous bluish *Paragonimus* cysts everywhere. These are most numerous at various points of adhesions, as between omentum and intestine or abdominal wall.	A mass of adhesions and dark, bluish cystic abscesses from 0.001 to 0.020 in diameter. Omentum thickened and simply studded with *Paragonimus* lesions. The pelvic cavity so massed with adhesions and abscesses that contents can only be removed with the knife.	Similar to Cases I and II
Diaphragm	On the left side much thickened and adherent above and below; contains many *Paragonimus* lesions on both surfaces.	Very much thickened and contains abscesses on the left side. Both surfaces studded with small, bluish cystic abscesses.	Similar to Case II
Spleen	Some perisplenitis with *Paragonimus* lesions at points of adhesion. Moderate increase of soft splenic pulp.	Enlarged and soft, contains both on the surface and in the parenchyma many bluish cystic abscesses containing ova.	Marked perisplenitis; organ hard and bloodless. Numerous subcapsular abscesses; deeper structure free from lesions.
Liver	Adherent to abdominal wall, diaphragm, intestine and omentum. Bluish *Paragonimus* lesions at points of adhesion. Multiple abscesses very small, many of which contain both amœbæ and *Paragonimus* eggs. Nothing abnormal in gall bladder or ducts.	Enlarged and rather soft, marked perihepatitis. Glisson's capsule increased and contains a *few* small abscesses. Numerous subcapsular abscesses. Gall bladder wall thickened and pale, surrounded and infiltrated with abscesses and one parasite found in common duct.	Chronic perihepatitis. Some increase of connective tissue with ova in places but no abscesses in deeper portions of organ. Numerous subcapsular absceses containing eggs. Gall bladder and ducts appear normal and are free from parasites or eggs.
Pancreas	A few small, bluish cystic abscesses near the head, mostly peripancreatic, but some invading the parenchyma of the organ.	Contains a number of small abscesses and is hemorragic for 0.05 on its splenic end.	Appears normal
Appendix	Bound in a mass of adhesions with several typical lesions involving the mesoappendix, walls and mucous membrane of the organ.	Walls and mesoappendix contain abscesses but mucosa intact except around the opening in the cæcum where there are several abscesses.	Walls and mesoappendix contain nodules and abscesses and mucosa ulcerated with ova in the scrapings.
Stomach	Not involved except in adhesions on the serous surface.	Not involved except by adhesions on the serous surface.	Appears normal
Small intestine.	Severely involved in its entire length. Typical cystic abscesses invading all its coats and ulceration of the mucosa.	Involved throughout. The walls contain many abscesses some of which protrude into the lumen of the bowel and others have broken down leaving ragged ulcers of the mucosa. There are a number of adhesive bands partly occluding the lumen.	Upper two-thirds appears normal; lower one-third similar to large intestine.
Large intestine.	In general very similar to small intestine and in addition shows amœbic ulceration of the mucosa throughout.	Walls much thickened and contain many cystic abscesses, some of which protrude into the lumen of the bowel. There is no ulceration of the mucosa.	Walls thickened and hard, dull, slate color, and contain many hard nodules and a few cystic abscesses. Mucosa ulcerated throughout. The ulcers are punched out and surrounded by zones of congestion. The contents dark in color and contain ova. In one an adult parasite was found.

eight fatal cases of paragonimus infection—Continued.

IV.	V.	VI.	VII.	VIII.
Free from adhesions except around the duodenum where there is a mass of old adhesions covering a post-peritonial *Paragonimus* abscess leading down behind the left kidney.	Extensive *Paragonimus* infection similar to Case II.	Adhesions and rather extensive *Paragonimus* infection of omentum and mesentery.	Moderate number of adhesions with rather extensive *Paragonimus* lesions particularly in omentum.	Tuberculosis. No *Paragonimus* lesions found.
Moderate thickening of the left side and a few cystic abscesses on the surfaces.	Extensive *Paragonimus* infection similar to Case II.	Moderate number of abscesses on left side on both upper and lower surfaces.	Two bluish cystic abscesses on under surface left side.	*Paragonimus* lesions left upper surface.
Appears normal	Not involved	Appears normal.	Slightly enlarged and soft.	Tuberculosis.
Moderately cirrhotic. Gall bladder and ducts appear normal.	Moderately firm and few subcapsular cystic abscesses.	Moderate fatty degeneration. No *Paragonimus* lesions.	------	------
Appears normal	Appears normal.	Appears normal.	------	------
Appears normal	Similar to Case III.	Appears normal.	Appears normal.	------
Appears normal	Appears normal.	Appears normal.	Acute catarrh	Tuberculosis.
Appears normal except duodenum which is bound down with adhesions.	Severe *Paragonimus* infection of lower one half.	In lower portion a small area of bluish cystic abscesses in wall.	*Paragonimus* infection throughout. Acute dysenteric lesions in lower ¼.	
Appears normal externally	Rather extensive infection similar to Case II.	Healing amœbic ulceration.	*Paragonimus* infection throughout, lesions of acute dysentery throughout.	Tuberculosis.

TABLE No. 4.—*Showing pathological summary in*

	I.	II.	III.
Kidneys	Both organs large, soft and œdematous. Adhesions and *Paragonimus* lesions around the kidneys. Lesions under the capsule and in pelvis and ureters. Adrenals appear normal.	Numerous adhesions and subcapsular abscesses. Adrenals seem normal.	Pale and soft; two small subcapsular abscesses and one pelvic abscess in left kidney. Adrenals seem normal.
Bladder	Walls contain several cystic abscesses.	Wall thickened and contains several abscesses, some extending into the lumen of the organ. The mucosa intact.	Appears normal
Scrotum	Epididymis is swollen and contains typical lesions which extend through the inguinal canal to lymphatics in abdominal cavity.	Epididymis contains several small abscesses.	Epididymis contains a few small abscesses.
Prostate gland.	Enlarged and on section contains several cystic abscesses.	Contains a few small, bluish, cystic abscesses.	Contains a few small abscesses.
Cerebro spinal canal.	Not opened	Not opened	Not opened
Miscellaneous	Ova or parasites found in most of the lesions mentioned but in some otherwise typical ones neither eggs nor worms were found. Amœbæ found in intestinal ulcers, liver, abscesses, in the prostate and in some of the lymphatic abscesses in the abdomen.	Ova and parasites in most of the lesions examined but not in all. This was apparently an acute case and no etiologic agent except *P. westermanii* was found. Death apparently entirely due to the fluke infection.	Many ova found extensively distributed. One psoas abscess contained more than 100 adult parasites.

eight fatal cases of paragonimus infection—Continued.

IV.	V.	VI.	VII.	VIII.
Appear normal	Appear normal.	Moderate cirrhosis.	Cloudy	
Appears normal	Appears normal.	Appears normal.		
Appears normal	Appears normal.	Appears normal.		
Appears normal	One small cystic bluish abscess.	Appears normal.		
Membranes of brain thickened and adherent in many places and have many cystic abscesses, containing ova in the masses of adhesions. Choroid plexus coarse and granular, slightly bluish and contains ova. No abscesses in the brain substance.	Nothing abnormal found.	Not opened		
The post-peritoneal abscess was evidently the oldest lesion in this case.	The double infection of tuberculosis and *P. westermanii* is the notable feature of this case.	The interesting feature of this case was the freedom from infection of the lungs.	The interesting point of this case is the complete absence of involvement of the chest organs by the *Paragonimus* infection.	Combined tuberculosis and paragonimiasis. The fluke infection confined to the chest organs.

C. GROSS LESIONS.

Rather liberal abstracts from the autopsy protocols in my eight fatal cases are given in Chapter V of this report, and these findings are epitomized in Table No. 4.

All the previously described lesions, as may be seen from a study of these cases and of the literature on the subject, are found in these eight and in addition new distributions and pathological processes caused by the parasite are recorded. In three of these cases the distribution is extensive enough to justify the term general infection; in two it is so markedly predominant in the lungs that *thoracic* or *pulmonary paragonimiasis* would seem a proper designation in these instances; in one case of epilepsy the term *cerebral paragonimiasis* would more clearly indicate the nature and most active location of the processes. Whatever the location or distribution of the lessions, there is always a great similarity between them.

These *Paragonimus* lessions have certain characteristics in general, which are sufficient to make it possible usually to identify the mature ones even without finding the parasites or without using the microscope. These characteristics are the color, general structure, contents and the absence of evidence of active inflammation.

The color of the lesions is a peculiar dull, bluish-slate, without evidence of pigmentation, and it seems to be in part due to reflection from the contents through the walls of the cyst, but this is not entirely the case for, after opening and cleaning a lesion, some of this color remains in the wall.

The general structure of the mature lesion is that of a necrotic abscess with a definite and more or less dense wall, which appears to be made up of layers. The outer surface is intimately connected with the surrounding tissues and the inner one is usually a smooth appearing membrane, but it may give evidence of being granular.

The contents vary but little. In addition to adult parasites, and eggs which may or may not be present, there is a material resembling anchovy sauce filling the entire cavity. At times (probably in mixed infections) the contents may appear more like ordinary pus and again it may be caseous and hardly distinguishable from a tuberculous lesion. It must be remembered that such a double infection may be present. As a rule there is an almost complete absence of the usual evidences of inflammation in or around these lesions.

While lesions answering to the description given above are characteristic, and while some of them may be found in every case of the disease, there are several important exceptions. These exceptions are so prominent that for convenience in discussion I will somewhat arbitrarily give the following classification:

- (*a*) The non-suppurating lesion.
- (*b*) The tubercle-like lesion.
- (*c*) The suppurating (abscess) lesion.
- (*d*) Ulcerative lesion.
    - Skin.
    - Mucosa.
        - Bronchial.
        - Intestinal.

(*a*) The *non-suppurating lesion* may or may not be an early one. It has three subtypes.

1. Those found on serous surfaces such as the pleuræ and, less frequently, the pericardium or meninges. These lesions are nearly always located at some point of adhesion between serous surfaces and consist in raised, brownish-colored, slightly roughened areas, which are moderately firm and which often contain ova. (See Pl. I.)

2. Those found in the loose connective tissue, for example in the subserous tissues of the intestine, and less frequently in the lung, liver and elsewhere. They consist in a simple infiltration of the tissue with eggs, without to any extent alterating the normal histological picture (see Pl. IX, fig. 1); sometimes there is seem moderate inflammatory reaction and in more advanced ones some increase in connective-tissue formation. These lesions are almost certainly early ones and immediately precede the ones about to be described.

3. Those which consist in a simple hyperplasia of connective tissue, containing ova. They are sometimes located in the cirrhotic liver and more often in the submucous and subserous coats of the intestine, and in the lung.

4. The pneumonic areas often encountered in the lung. These pneumonic areas closely resemble similar lesions from other causes and may or may not contain parasites or ova. They probably, at least in part, follow lesions of types 2 and 3.

b. Tubercule-like lesions most often are found in the lung, but occasionally are also observed in other organs.

Miura and other observers described such lesions as closely resembling miliary tubercles, but I have not found such exceedingly small and definite ones of this type. However, in the lungs and elsewhere caseating nodules are encountered which are from 2 to 10 millimeters in diameter and which are almost or quite indistinguishable from similar ones of tuberculous etiology.

c. The suppurating lesions.—These are of two kinds, the first showing early formation of abscesses, which in appearance resemble ordinary pyogenic ones, probably followed at times by the second kind which is characterized by the development of a cyst wall. However, this is not the universal course of these lesions, for sometimes, particularly in the prostate gland and in the lymphatic glands, the ordinary type of abscess formation is more nearly maintained during the course of the infection and the cyst wall, with the bluish coloration, does not develop. Again, we find the more cystic type of development shown in some of the smallest and earliest lesions. While the ordinary lesions which appear like abscesses often contain ova, their presence can hardly be said to be characteristic of the disease, and this type of lesion is probably due, in part at least, to other ætiologic influences. The bluish-slate colored, cystic, abscess-like lesions are more or less frequent in every case. They are characteristic of the disease and have already been described.

d. Ulcerative lesions are found both in the skin and mucous membranes. In both cases they are the result of perforation of lesions from the lymphatics and other underlying structures and are probably never the result of direct infection of the skin or mucosa. They are somewhat distinctive, of a slow chronic type without much acute inflammatory reaction. Their edges are often of a bluish color, and are overhanging. Their contents is of a granular nature and the opening leads to the underlying tissues. The skin ulcers most often are found in regions rich in superficial lymphatics such as the groin or axilla, and the ulcers generally communicate with infected and broken down glands. In one of my cases, two ulcers in the groin had been diagnosed and treated as "tropical ulcer." Microscopic examination of the contents of the lesions at autopsy showed the eggs of the fluke under discussion.

The ulcers of the mucous membrane are most often seen in the bronchi and in the intestine, less frequently in the bile ducts. When they are in the bronchial tubes they usually communicate with bronchiectatic abscesses and the primary lesion is in the deeper structures, as has already been described.

In the intestine, also, the primary lesion is in the deeper layers, and the breaking down of the mucous membrane with ulcer formation is a secondary result. I have not often been able to find eggs in the mucosa, but the deeper structures concerned in the ulcer may have many eggs and sometimes adult parasites. This is in marked contrast to the *Schistosoma* infection, where, for example, the mucous membrane suffers most actively and the eggs are numerous in this part of the bowel.

The intestinal ulcers are usually of irregular shape and do not bear a definite relation to the mesenteric border or other anatomical structures of the bowel. In well-advanced cases they may quite closely resemble tuberculous or amœbic ulceration, particularly when bismuth salts have been administered in the latter cases and the edges of the ulcers have been darkened by impregnation with these chemicals; this

I have pointed out in previous papers in respect to tuberculosis and amœbic dysentery.

Course and termination of the lesion.—The several types of departure from the earliest point of infection have been described, and all of these may be found in a case of extensive infection at the same time. The secondarily infected, or mixed lesions, and the process of healing which is sometimes seen may also be considered here.

Mixed lesions.—These probably occur quite frequently and they may be of several kinds. In one of my cases many of the abscesses in the liver and other organs contain amœbæ and *Paragonimus* and in two others the intestinal lesions were of this double infection. In addition to this, bacteria have been found, and while this subject has not been sufficiently investigated, it is probable that this type of mixed infection is of great importance. In case 2, which apparently pursued a very rapid course, numerous bacteria were found in the *Paragonimus* lesions and in one other the abscesses of the prostate were more of a pyæmic nature than they were of a parasitic type, although the trematodes and the bacteria were present. It would seem that what at first appeared to be a single infection and which seemed to establish for the parasites the capability of inaugurating an acute process, probably constituted in reality a double one, and that the evidences of the more acute inflammatory changes were due to the bacteria present.

Healing to the extent of complete disappearance of all evidence of the former infection probably does not take place, but by encapsulation and other connective-tissue hyperplasia the lesions may become inactive so as to produce no further disturbance. I have several times seen areas of old, bluish scar tissue which, from the amount of contraction present, probably resulted from what formerly constituted rather extensive, *Paragonimus* lesions.

D. SPECIAL GROSS PATHOLOGY.

It seems advisable at this point, after discussing the general pathological anatomy and the lesions of this infection, to introduce a brief explanatory résumé of the pathological findings which are shown in Table No. 4.

The *conjunctivitis* noted in 2 of the 8 cases was probably not due to the fluke infection. In one of these, a careful microscopic examination was not made and in the other such examination did not show ova. Similar types of severe conjunctivitis, largely of the Koch-Weeks variety, are of very frequent occurrence among the prisoners from which much this material was obtained.

Skin lesions, consisting of ulceration, were present in 2 out of 6 of the fatal cases. These ulcers in each instance communicated with diseased lymphatics and their characteristics have already been given.

Lymphatics.—Both the subcutaneous and deeper lymphatic glands suffered in at least 5 out of 7 of the 8 fatal cases. Their involvement is much more extensive and important than is indicated in the literature of the subject. In one case (Pl. 2, fig. 2) an adult parasite was found in an abdominal gland. All types of lesions are observed in the glands and in some, where there are no distinctive abnormal features, ova may occasionally be found. The lymphatic disturbance is usually so extensive as strongly to point to the lymphatic system as the distributing agent of the disease in the body.

Muscles.—Adult *P. westermanii* were found in the pectoralis major muscles in 2 of the 8 cases and in 3 the psoas or other muscles contained abscesses.

Pleuræ.—The pleuræ were infected in 7 of the 8 cases, and in most of them the infection was rather severe and extensive. Both hydrothorax and extensive obliterative pleuritis were encountered.

Lungs.—The lungs were absolutely free from *Paragonimus* infection in one of the 8 cases and in 1 it was confined to the lungs and pleuræ, and in still another the pleuræ was involved without invasion of the lungs. The lung lesions, consisting of pneumonic areas, abscess formation, infiltration of tissue, and bronchiectatic cavities, have already been described.

Pericardium.—Pericarditis, with eggs of the *Paragonimus* in the pericardium was present in 4 of the 8 cases.

Heart.—Brown, coarse and friable muscle was present 3 times and *Paragonimus* involvement extending a short distance beneath the epicardium was encountered in the same cases.

Abdominal cavity.—The tissues and organs of the abdominal cavity usually suffer severely in this disease.

The *omentum* was involved in 6 of the 8 cases and in most of these the distribution of the lesions was extensive.

Chronic peritonitis, consisting of adhesions between adherent adjacent organs, was usually a marked feature. The serous capsules covering the various organs also seemed to suffer extensively.

Diaphragm.—This organ was more or less involved in every case and in some it was fairly studded with lesions. In one instance only the pleural surface on the left side was involved and in another only the abdominal surface showed lesions.

Spleen.—*Paragonimus* lesions were scattered throughout the organ in one case; the capsule was similarly involved in two more and in one there was tuberculosis of the organ.

Liver.—There were changes for which the *Paragonimus* infection was at least partially responsible in 6 of the 8 cases. The most general disturbance was perihepatitis, with abscesses just beneath the capsule. In one, there were multiple abscesses containing both amœbæ and ova of *P. westermanii.* There were abscesses containing eggs in Glisson's capsule in one other case and in 2 more there was some cirrhosis with eggs in the hyperplastic connective tissue.

Pancreas.—In 2 cases there were *Paragonimus* abscess lesions in the pancreas.

Appendix.—There were parasitic lesions of the appendix in 4 cases. The meso-appendix suffered most, but in 3 the entire organ was affected and in 2 there was ulceration of the mucosa.

Stomach.—This organ was not involved in any instance with the exception of a few ahesions on the serous surfaces in 2 cases.

Small intestine.—There was infection of this organ in 6 of the 8 cases. In 3 the infection was severe and extended the full length of the gut. In 1 only the duodenum was concerned and in 3 others only the lower portion was affected.

Large intestine.—This organ was affected in 5 of the 8 cases. Tuberculosis was present in one instance. Amœbic and *Paragonimus* ulceration existed together in case 1 and there were amœbic lesions alone in another.

Kidneys.—The kidneys were included in the infection three times; the lesions were mostly closely connected with the capsule, but in 2 there were abscesses of the pelvis and in 1 the ureters were included in the affection.

Urinary bladder.—This organ had *Paragonimus* abscesses in the walls in 2 cases.

Scrotum.—The epididymis contained parasitic lesions in 3 instances; in 1 the infection extended through the inguinal canal to the abdominal lymphatics.

Prostate gland.—The prostate was involved four times. The lesions were of the abscess type and contained ova.

Brain and spinal cord.—These organs were only examined twice. In one instance there were *Paragonimus* lesions of the membranes of the brain, and eggs were found in the choroid plexus.

E. HISTOLOGY.

The literature bearing upon the microscopic study of the disease principally consists in the description of two types of lesion—the tubercle and the bluish-colored necrotic abscess.

The older literature is well summarized by Stiles and Hassall; since that summary was compiled, some original observations have been made, particularly by Katsurada. Looss has recently given another brief summary in Mense's handbook.

I shall discuss the lesions under the same classification as that already used in describing the gross pathology. They have certain points in common. In all the strictly parasitic types of infection there is the evidence of a slowly developing, low grade of chronic inflammation, with constant effort at repair which is chiefly manifested in the extensive proliferation of the connective-tissue framework, the new tissue being deposited about the original lesion, gradually encapsulating it or in some other manner tending to prevent its further spread.

This is made most manifest in the fully developed cystic abscess lesion, where the limiting wall is often very firm and thick. In other types it is less evident and in some of the very early infiltrations of the loose connective tissue with eggs it is entirely absent.

Eggs or parasites are by no means constantly present in any variety of the lesions. In the earlier forms the diagnosis can only be made by discovering the presence of these bodies, for there is nothing else characteristic about the lesions. The older ones and particularly those of the abscess variety take on characteristics which should be diagnostic even in the absence of eggs or parasites. Again, the greatest variation often exists in the number of eggs or parasites in a given lesion, sometimes the eggs are very numerous, and on the other hand, in an otherwise exactly similar lesion they may be very scarce. Parasites when present may range from one to many. More than 100 adult parasites were found in a psoas abscess.

The *nonsuppurative* lesions are of several types; however, some of these are but later stages of others, but there are certain ones which differ in formation as well as in their entire course.

The loose connective tissues, particularly in the subserous coat of the bowel, and less frequently elsewhere, may sometimes be found infiltrated with eggs without any decided change in the histology of the tissues themselves (Pl. X, fig. 1); however, in a later phase of this condition two changes begin to take place—a proliferation of connective tissue and beginning round cell infiltration. As the connective tissue wall developes in quantity, eggs may be surrounded by it, the proliferated cells increase in numbers and eventually the next stage is recognized—cirrhosis—or the lesion begins to break down and an abscess is formed. The early changes above referred to are rarely seen in the liver, but the second stage, with moderate cirrhosis by increase, particularly in the larger bands of Glisson's capsule, was present in one of my cases. After an examination of many specimens I have found a few eggs in apparently nonproliferating tissue of the liver.

The histology of broncho-pneumonia, another nonsuppurative type of lesion, does not differ materially from similar lesions due to other etiology. The exact method of formation is difficult to explain, but inasmuch as the two first stages of infiltration mentioned above are also seen in the lung, and bearing in mind the part played by the lymphatics in the distribution of the eggs, it would seem that the pneumonic areas, at least in part, may follow some of the other lesions.

Pneumonic areas sometimes become converted into the abscess type. The amount of embolism and thrombosis in this disease is exceedingly small. Lesions of the serous surfaces may be of the ordinary type, but often they have certain distinctions. They are most numerous about old adhesions, which may or may not be of *Paragonimus* origin. The adherent surfaces when separated are rough, raised, often of a brownish color, and contain eggs; sometimes these lesions break down and abscesses are formed. The histologic picture in these types shows a fibrosis, with very moderate round cell infiltration. Here, as in the other types discussed above, there is sometimes, but not often, a marked eosinophilia present.

Tubercle-like lesions, particularly those very much like miliary tubercles, have been mentioned by several writers. Miura states that they correspond histologically with Virchow's "fibrous tubercles," the centers containing eggs and often giant cells. These lesions have been considered the primary ones and all others the result of coalescence and changes in the tubercles.

Although lesions closely resembling more advanced tuberculosis are often encountered in these cases, I have not seen the type of miliary tubercle except in one case, and here the lesions were of true tuberculous origin. To judge from the descriptions of some of the cases in literature, it would seem that mixed infections such as were present in this case had been described. As has already been stated, eggs and tubercle bacilli have been found together in more advanced types of lesions and, as is true in some other parasitic diseases, the infection seems to become more active in an already established lesion, let us say of tuberculous origin.

The abscesses are the characteristic lesions and they have been described in almost every article dealing with the disease. In brief they consist of three zones, the central one made up of necrotic tissue, degenerated cells and often of eggs or fragments of eggs; the middle coat consists of a more or less thick and dense fibrous capsule, formed of proliferated connective tissue, infiltrated to a moderate degree with round cells and sometimes with eggs. Its inner wall may be smooth and lined with epithelial cells, but it is often granular. The outer zone is one of moderate congestion, with proliferation of blood vessels and it is continuous with the surrounding tissues.

However, there is another type of abscess encountered most often in organs such as the prostate, epididymis, and the lymphatic glands, which histologically as well as in gross appearance more closely resembles an ordinary pyogenic abscess, and were it not for the presence of eggs or parasites such would surely be the diagnosis in many of these cases.

Ulcerative lesions are formed by the breaking through the mucosa or skin of any of the types already discussed, as a result of pressure, necrosis, thrombosis, or otherwise. This most often happens when the underlying lesion is an abscess, but it may take place with other types. In the intestine, the striking fact about these lesions is the usual absence of eggs or parasites in the mucosa, whereas the submucosa and deeper structures often contain them in large numbers and discharge them through the opening into the lumen of the bowel. This relative immunity of the mucous membranes to *Paragonimus* infection has already been mentioned, and it is in striking contrast to that with *Schistosoma* for example, where the mucosa is often infiltrated with eggs.

In the lungs this ulcerative process is probably the mode of formation of the bronchiectatic cavities. This is more probable than the belief that they result from a direct attack on the mucosa, or that they follow occlusion of a bronchus. In either case, the bronchial wall can and sometimes does take part in the formation of the abscess wall, but this, as has been emphasized by some writers, is not the rule. A moderate peribronchitis of quite general distribution in the smaller tubes is quite common in extensive lung involvement, but is more the result of the chronic bronchitis than of a *direct* influence by *P. westermanii.*

In the intestine, just as in the lung, the deeper structures and coats seem to be primarily involved, and the infection spreads through the subperitoneal muscular and submucous coats, often finding an outlet by ulceration of the mucosa. Frequently, however, ulceration does not take place even when tumors of the mucosa are pushed into the lumen of the bowel. Sections of one of this variety of lesion show a thickening of the connective-tissue layers, with proliferation of the fixed cells and sometimes a marked eosinophilia. Mixed infection in any type of lesion may alter its histology very much, this alteration depending upon the nature of the secondary invader.

VIII. SYMPTOMATOLOGY.

A. GENERAL DESCRIPTION.

The clinical observations upon this disease have been largely confined to the symptoms of the pulmonary and cerebral types of the infection, and for these a summary of the literature gives a satisfactory picture of the disease as I have seen it. However, a study of the material upon which this report is based shows the disease to be much more generally distributed in the body than formerly it has been recognized to be, and therefore it necessitates a more comprehensive clinical picture than any which has previously been given. However, before taking up a consideration of these special types more in detail, it is necessary to discuss the general type of the disease in regard to its *acuteness.* Heretofore, it has been considered as being almost wholly a *chronic* infection, but some of my cases have shown a most acute and rapid course.

ACUTE AND CHRONIC PROCESSES.—It is probably a fact that the true and uncomplicated *Paragonimus* disease is always a chronic one, and that the more acute processes are the result of some concomitant infection or complication. This has already been pointed out as probably accounting for the rapid course of some of my cases and it is a condition which needs more study based upon a larger material. However, it is a fact that we must consider from a clinical standpoint at least some of these cases as acute, and while this condition obviously would be more likely to occur in a general infection, it surely may take place and influence the course and termination in some of the more restricted types.

B. CLINICAL TYPES.

After a careful study of the literature and of my own material, as well as I have had opportunity to do so, it seems advisable to consider the symptomatology under the following *arbitrary clinical* classification:

(*a*) Generalized paragonimiasis.
(*b*) Thoracic paragonimiasis.
(*c*) Abdominal paragonimiasis.
(*d*) Cerebral paragonimiasis.

(*a*) GENERALIZED PARAGONIMIASIS.—A full consideration of the general infection would produce a composite picture of the other more local varieties, which I have already given in the classification and therefore, in order to avoid repetition, only those points in the clinical symptoms which are not directly covered in the discussion of the other clinical types will be noticed here. Some of the most interesting of these symptoms are produced by involvement of the superficial lymphatics and the skin. Enlarged lymph glands are quite common among the people of this country, and cases of so-called "glandular fever" and of "climatic bubo" are not uncommon; some of these, as well as certain of the so-called "tropical ulcers," may have their etiology in trematode infections. The symptoms produced by glands infected with trematodes are not different from those of similar lesions of other etiology, and an exploratory incision may or may not reveal the presence of eggs.

The skin ulcerations have nothing characteristic. They are probably secondary to the infected lymphatics beneath. I found eggs in the contents of the ulcers in two of my cases, and the older and more advanced ulcers have a slate-colored, overhanging margin. The lack of evidence of acute inflammation about such ulcers is important.

One of my fatal cases, while in the hospital, complained of muscular rheumatic pains, and similar symptoms have been pointed out by other writers; this is at least partially explained by the distribution of the lesions, parasites and eggs in the muscular tissue.

Fever, which has not previously been considered an important symptom in these cases, was high in 4 out of 9 of mine and it probably will be found to be a somewhat constant symptom in extensive infections and in mixed ones which run a rapid course.

(*b*) THORACIC OR PULMONARY PARAGONIMIASIS.—According to the literature this is by far the most frequent type of the disease and locations of the infection other than in the thoracic cavity are considered to be secondary to this primary focus.

In two of my fatal cases and in two more of the clinical ones, the lungs were not involved and in several others the older and more severe lesions were surely present in the other organs. The pleuræ, diaphragm and other chest structures are so commonly, indeed almost constantly, involved with the lungs that thoracic paragonimiasis seems to be the more satisfactory term for this class of infection.

The most prominent symptoms ascribed to this type comprise: Pains in the chest; cough with occasional small hæmorrhages, the sputum containing eggs and according to Taylor and others sometimes an adult parasite. The physical signs are usually considered as being slight. Cough is a prominent symptom in most of these cases, but in one of mine it was so slight as to attract but little notice. The nature of the cough is as variable as that observed in tuberculosis, and the character and appearance of the sputum has an equally wide divergence; both depending largely upon the stage or nature of the underlying pathological change and the amount of lung tissue involved, together with the condition of the pleuræ and heart membranes, this condition depending upon the presence or absence of fluid, adhesions, etc. I have also convinced myself that the finding of eggs in the sputum in the average case requires more time and care than is usually supposed to be the case. Indeed, in one instance eggs were not found after several careful examinations made for this specific purpose, and yet at autopsy the lungs and pleuræ were found to be involved. The number of eggs in the sputum depends upon the extent and particularly upon the *character* of the lesions. Just as is the case in tuberculosis, it is obvious that more of the eggs will be discharged from bronchiectatic cavities than from the equally important pneumonic areas. With this in view it seems hardly likely that *early* diagnoses are probable in this disease, and the need of *repeated* and careful search for eggs in the sputum of suspected cases is also emphasized. Hæmoptysis has not been a constant symptom in my cases, at least while they were under observation, but in one instance it was repeated and quite severe. In a country where tuberculosis is so very general, hæmoptysis would call attention to the disease under discussion only in the absence of other tubercular symptoms. Charcot-Leyden crystals are at times quite numerous in the sputum or again may not be observed at all. Other types of crystals, such as those of creatinin are also sometimes present. When the pleuræ are extensively involved, with but slight or no extension of the disease into the lungs, the chances of finding eggs in the sputum will be very small and the diagnosis will then have to be made largely from physical signs. The fluid from a *Paragonimus* empyema contains very few eggs and therefore, when this is the case, aspiration of the pleural cavities would often fail in furnishing a diagnosis.

In two cases under my observation which had pericarditis, the manifestations were similar to those encountered where this disease is of other etiology.

The physical signs in thoracic paragonimiasis have a greater importance than is usually ascribed to them. Bronchopneumonia, empyema, hydrothorax, etc., due to this infection show the same accurate physical signs as they do in the similar conditions produced by other causes. In one clinical case the presence of an empyema with pericarditis led to a careful examination of the sputum, and this determined the diagnosis. It must not be forgotten that, particularly in Manila, tuberculosis will be found associated with *Paragonimus*.

(*c*) ABDOMINAL PARAGONIMIASIS.—This term is used by me to include the infection of any or all of the abdominal organs.

The abdominal infection is usually a rather extensive one and will hardly admit of a positive diagnosis without an exploratory operation or the finding of the eggs in the fæces. Eggs are probably only found in the fæces in the presence of ulcerative lesions in the bowel. In the several cases which I have had the opportunity of studying there was abdominal pain, mostly of a dull, aching character; in such instances the abdominal walls may be rather hard and may show some tenderness on pressure. In areas where the infection is endemic, hypertrophy of the prostate, cirrhosis of the liver, chronic epididymitis or lymphadenitis of doubtful etiology, should always lead to a careful study in which fluke infections are taken into consideration. If there is ulceration of the intestinal mucosa, the eggs may be found in the fæces and there may or may not be diarrhœa. This fluke may possible at times be the etiologic factor in chronic appendicitis, but the infection will hardly be found confined to this organ.

(*d*) CEREBRAL PARAGONIMIASIS.—This important type of paragonimus infection was first recognized by Otoni and Inouye and since that time has received considerable discussion, particularly in its relation to Jacksonian epilepsy, of which it is one of the causes. Other prominent nervous symptoms consist in various forms of neuritis and even paralyses have been observed in this infection. Unfortunately, the brain and cord were removed in only two of my cases—in one because epilepsy was a prominent symptom during the short time the patient was under observation.

C. PARAGONIMIASIS OF THE LOWER ANIMALS.

P. westermanii is known to infect the tiger, cat, dog and hog, but as has been pointed out by Stiles and Hassall, very little is known of the symptomatology and pathology of the disease in these animals. Since the discovery about one year ago of the first case of this infection in man in the Philippine Islands, the following animals have been carefully examined for fluke infection:

Thirty-two cats with 1 positive result for *P. westermanii* and in 17 others *O. felineus* was encountered; 23 dogs with no case of infection with *P. westermanii;* 10 rats and mice with no case of fluke infection of any kind.

Paragonimiasis of the cat.—The case of one animal in which this infection was encountered in the Philippines is interesting. The animal was purchased from a native and was placed in a large cage with several others and left for about one week. When received it was rather thin, but otherwise apparently healthy. At the end of a week it was brought to me by the animal care-taker in a dying condition. Emaciation was marked, food was refused, there was diarrhœa and partial paralysis of the hind quarters. The animal was chloroformed and autopsy performed at once. *Paragonimus* lesions with eggs and parasites were found in the lungs, intestines and omentum. The lesions were not extensive and both these and the parasites were apparently identical with those found in man.

IX. DIAGNOSIS.

A direct, positive diagnosis of paragonimiasis can only be made by the finding of parasites or eggs. Under favorable conditions the eggs may be encountered in the sputum, fæces, scraping from ulcers, and in fluids and tissues removed by operation.

The sputum should be examined as a cover-glass preparation of a fresh specimen and, in suspected cases, the examinations should be frequently and carefully made during considerable periods of time. The eggs are easily recognized under the lower powers of the microscope;

their outlines may be made sharper and clearer and the operculum may be rendered more distinct by adding a small quantity of dilute sulphuric acid to the specimen, or by running a drop or two of a 0.1 per cent solution under the cover glass. The ordinary munipulations used in preparing a specimen of sputum for examination for tubercle bacilli either destroy the eggs or reduce them to an unrecognizable condition. Once, in examining a slide for tubercle bacilli, peculiar appearing bodies were noticed, which led to the examination of a fresh specimen and to the recognition of a case of paragonimiasis which had previously been unsuspected. This patient also had tubercle bacilli in his sputum. While in several conditions Charcot-Leyden crystals are found in the sputum, they are in this disease more numerous and constant than the eggs of the parasite, and when seen in considerable numbers in a sputum from a patient in regions where the infection is endemic, their presence should lead to a closer investigation for *Paragonimus* disease. In the Philippine Islands, and other endemic zones, examinations of fresh specimens of sputum should be as much a part of routine as is the examination of stained ones.

Fæces.—Several times duirng the past year the presence of *Paragonimus* infection has been discovered by finding the eggs in the fæces. In one case the diagnosis was confirmed by an autopsy. In two of these instances no eggs could be discovered in the sputum. Only a simple cover-glass preparation is needed for the examination of the fæces and the eggs are most quickly located with an AA objective and a number 4 occular. The eggs are less numerous in the fæces than they are in the sputum, and they usually are found only after persistent search.

The primary lesions in the intestine are usually in the deeper layers and the eggs probably do not appear in the fæces unless there are ulcerations of the mucosa; this fact has been pointed out in the discussion of the pathology of this disease.

Ulcers.—Ulceration of the skin of a chronic type leading into deeper tissues should indicate careful examination of the scrapings for eggs of *P. westermanii.*

Fluids and tissues removed by surgical operation in obscure cases should be carefully scrutinized for eggs and parasites in zones where the disease is endemic. This is particularly true in operations which require the opening of the abdominal or pleural cavities.

In exploratory, diagnostic operations in the Philippine Islands, particularly upon the chest, abdomen, liver, scrotum or prostate, fluke infections should be kept in mind. The difficulty of distinguishing the egg of *P. westermanii* from some of the other fluke eggs has already been noticed in another part of this paper and need not be repeated here.

While, as stated above, positive diagnosis can only rarely be made in the living subject except by finding parasites or eggs, there are several subjective symptoms and physical signs which furnish strong supporting data. Of these the following, occurring singly or together, may be noticed: Unexplained epilepsy or other evidence of localized brain lesions; cough with occasional small amounts of blood, particularly with but slight or no constitutional disturbance and with broncho pneumonia and empyema. Evidences of chronic peritonitis, enlarged prostate, epididymitis, or cirrhosis of the liver, and chronic skin ulceration with enlargement of the lymphatic glands.

Owing to the slow progress of the disease in many cases and to the probable late appearance of ova in the excreta, many of these infections will continue first to be diagnosed at autopsy, until other accurate diagnostic data are available.

X. COURSE, DURATION AND PROGNOSIS.

The course is usually chronic, the disease lasting for years; however in some cases, and particularly in mixed infections, it may be acute, and death may result in a few days from the time the symptoms are first noticed. Several writers have stated that patients may live for fifteen or even twenty years.

The prognosis, if the patient remains in the region where the infection is endemic, is usually bad as to recovery, although several writers say that this may take place. After a study of the pathology of the disease, this statement seems difficult to believe. Evidence is sometimes found indicating that there may be an arrest of the progress of the disease, and were this to occur in the early stages of the infection, the patient might remain in good condition for many years. However, with the usual types of the disease it is doubtful if complete healing of the pathologic process ever takes place.

Death probably most often is caused by intercurrent diseases, but in at least two of my fatal cases no evidence of such a condition was found at autopsy.

XI. COMPLICATIONS.

While it is probable that a great variety of diseases may be found associated with paragonimiasis and while other diseases often develop during the course of this infection and hasten its outcome, there are but two which, because of their special importance, will be noticed here. These are tuberculosis and amœbiasis.

Tuberculosis, either local or general, may complicate either a local or more general distribution of the parasitic disease. The two diseases existed together in 2 of my 8 fatal cases and such a combination has in addition been found 3 times clinically. There are several reasons why this particular complication should receive close attention. The symptomatology of the two diseases is often very similar. The lesions are sometimes rather difficult to distinguish, and the two infections may be found co-existent in the same lesion.

Amœbiasis complicated 3 of my fatal cases, and the two diseases have been found together twice clinically. The association is particularly interesting in case 1, where both etiologic agents were found together in the multiple abscesses of the liver and in lesions in several other parts of the body.

It is probable that in intestinal paragonimiasis eggs would more readily be found in a bowel already ulcerated by amœbæ. The symptomatology of the two diseases, when the abdominal organs are particularly involved by the *Paragonimus*, may be very similar and the double infection easily overlooked.

XII. PROPHYLAXIS.

As has been pointed out by Stiles and Hassall, Looss and many other writers, no satisfactory prophylaxis against this disease can be established until the life cycle of the parasite has been elucidated. General hygienic usages are recommended by most authors, these measures, for example, being the use of cooked food and sterilized water and the disinfection of the sputa of infected hosts. In addition to the lack of knowledge of the complete life cycle of the parasite, successful prophylaxis is further inhibited by an ignorance of the complete list of the hosts of the adult fluke in zones where the parasite is endemic and its prevalence in these different species of animals.

Practically all writers have assumed that the infection takes place through the gastro-intestinal canal, and this is probably true, but it seems that the question of possible infection through the skin should also receive careful consideration. The frequent, extensive involvement of the lymphatics and the rôle played by this system in the distribution of the infection in the body of the animal host make it very important that skin infection be excluded in considering the manner of entrance of the parasite. The probable similarity between the life cycle of this fluke and that of some others with known life histories, adds another good reason for forbidding the use of uncooked food, particularly vegetables and water, in countries where the disease is endemic. For similar reasons it seems justifiable particularly to warn against the consumption of uncooked fish, clams, crabs, oysters and other shellfish obtained from local waters. From data brought out in this report it seems wise to recommend not only the destruction of the sputum of infected human hosts, but an equally careful disposition of the fæces and, in cases where ulceration of the skin exists, careful disinfection or destruction of the dressings should be a routine procedure. Finally, it seems that the spread of the disease might be somewhat limited by the destruction of as many of the lower animal hosts as can be discovered. In this country cats, dogs and pigs are very numerous; they enjoy unusually close relations with members of the community, and in the absence of a satisfactory means of the disposal of their excreta these must always prove a menace in infecting the probable harborers of the other phases of the life cycle of the parasite.

XIII. TREATMENT.

No form of treatment so far established is specific against the infection. Many different types of therapeutics have been used and some of them, such as inhalations of antiseptic substances for the pulmonary type, have been thought to benefit the patient.

Taylor recommends rest in bed during times where there is hæmoptysis or when the cough is very severe.

A general, tonic treatment, consisting both of good hygienic measures and drugs, is recommended by all observers and a change of climate to noninfected regions urged by some.

In one of my cases the cough disappeared and the general condition of the patient improved very much under large doses of potassium iodide.

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ILLUSTRATIONS.

PLATE I. Heart showing adult parasite (at *a*) and the raised brownish colored areas infiltrated with eggs described in the text.

II. Fig. 1. Lung showing pneumonic areas, one early abscess lesion, section of adult parasite in a bronchus and another in a pneumonic area.

Fig. 2. Lymphatic gland, showing necrotic lesion from which adult parasite was removed.

III. Adult *P. westermanii*, showing subterminal oral sucker, acetabulum, genital pore, excretory pore and intestinal cæca.

IV. Fig. 1. Egg of *P. westermanii*, showing cellular contents; operculum not in good focus.

Fig. 2. Fresh smear from *Paragonimus* abscess of spleen, showing eggs.

V, figs. 1–9, and Plate VI, figs. 1–9, represent 18 selected specimens from serial sections of the adult parasite cut longitudinally. Sections of all the important structures may be seen in these figures.

VII, figs. 1–9, and Plate VIII, figs. 1–9, are similar to Plates V and VI, except that in the former the sections are cut transversely.

IX. Fig. 1. From the subserous coat of the intestine, showing numerous eggs; otherwise but slight change in the tissues. × 35.

Fig. 2. From intestinal wall, tissue containing eggs, with cellular infiltration. × 35.

X. Fig. 1. From the intestinal wall, showing connective-tissue hyperplasia, cellular infiltration and necrosis, with many eggs.

Fig. 2. Typical, small necrotic lesion removed from lymphatic gland. × 46.

XI. Figs. 1 and 2. Both figures show sections through large abscess lesions containing adult parasites.

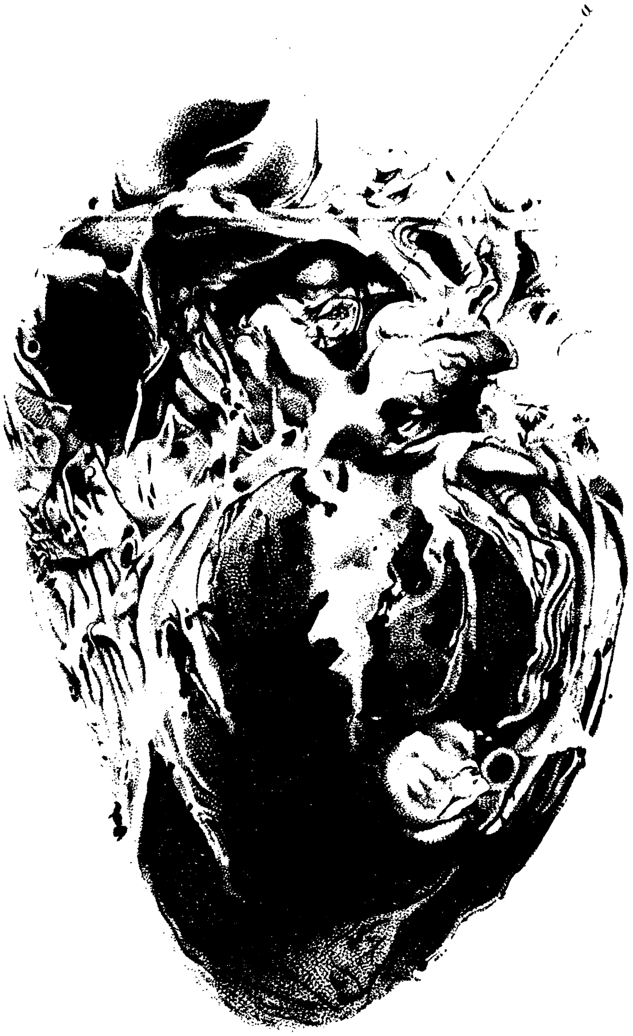


PLATE I.

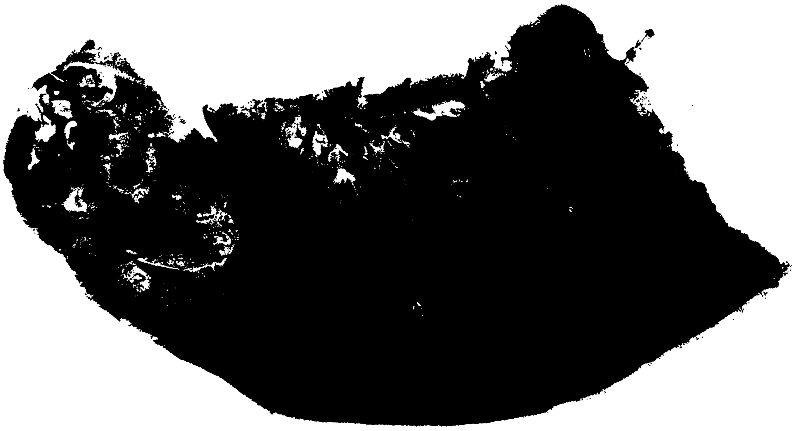
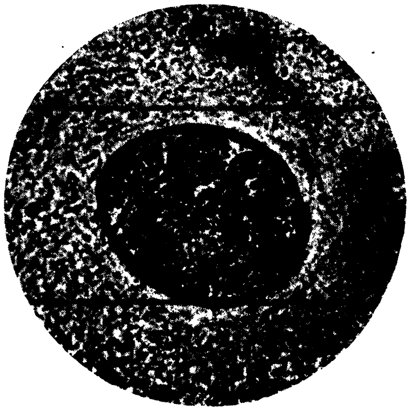


Fig. 1.

Fig. 2.

PLATE II.

PLATE III.



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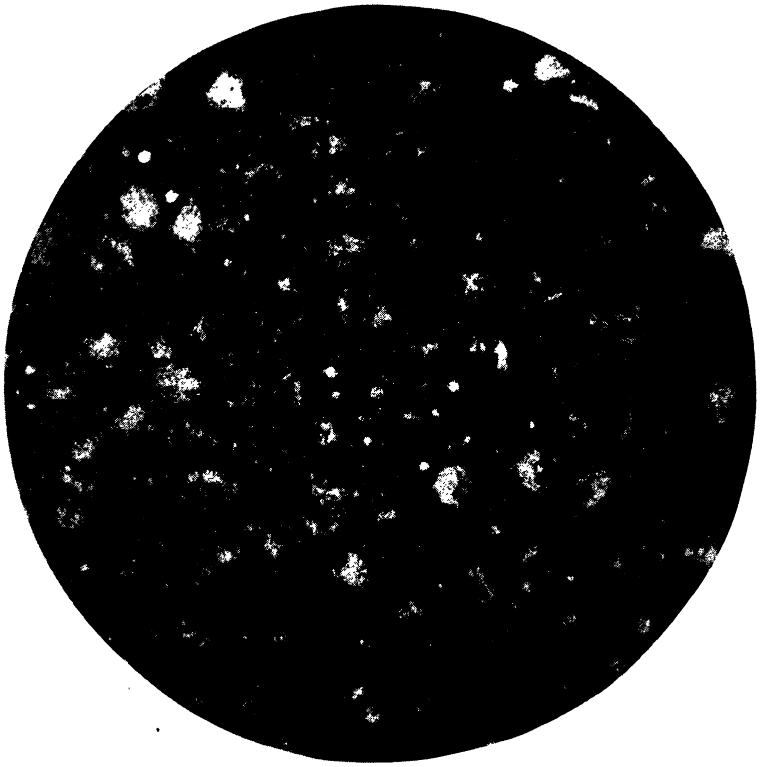
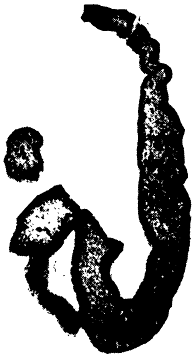


FIG. 2.

PLATE IV.



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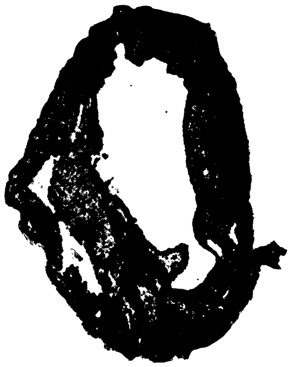

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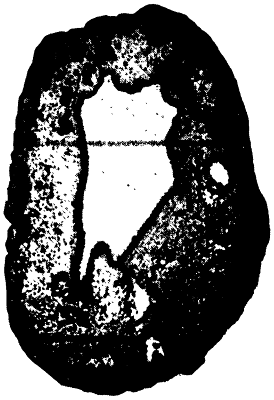

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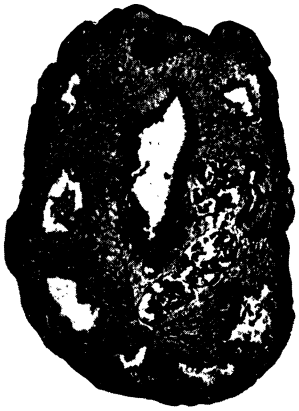

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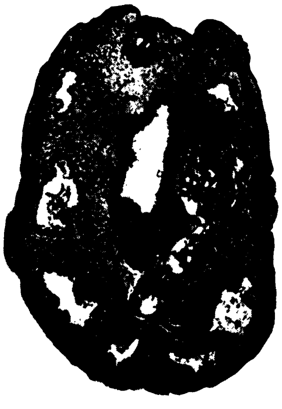

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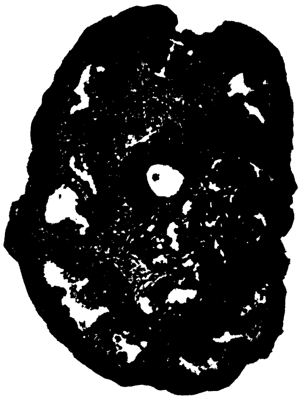

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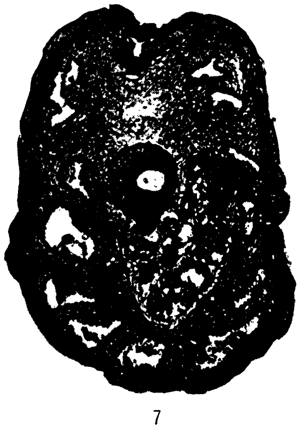

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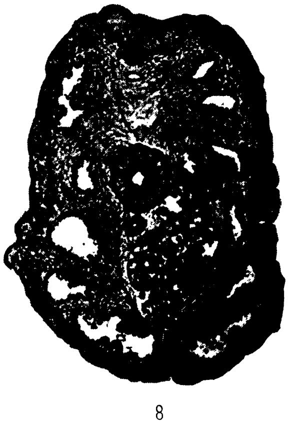

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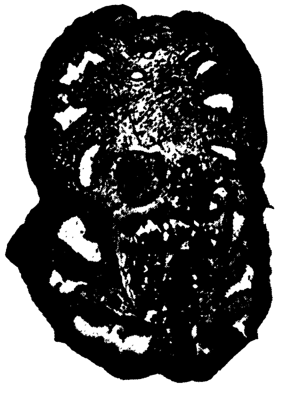

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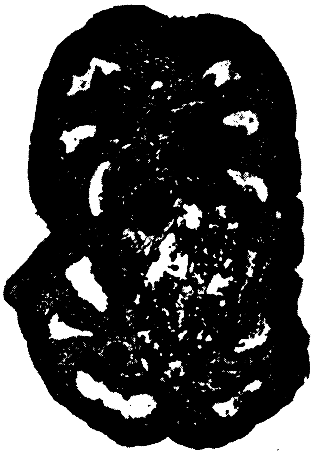
PLATE V.



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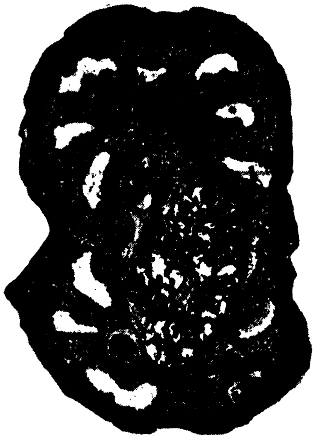

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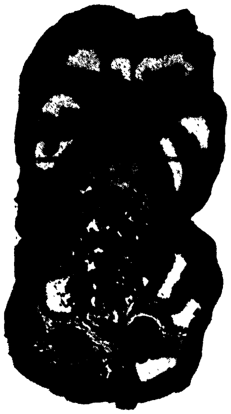

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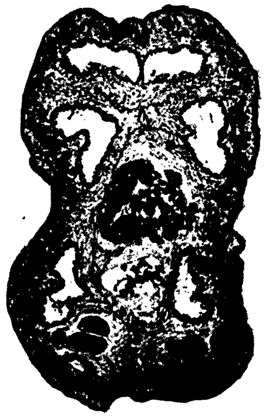

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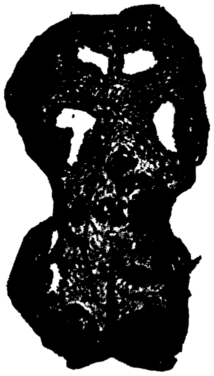

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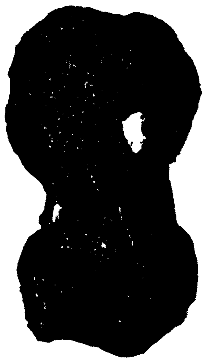

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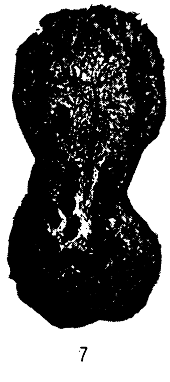

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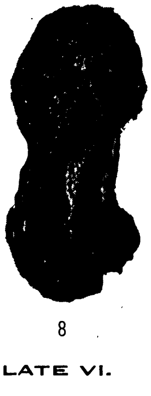

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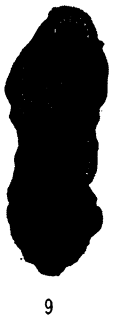

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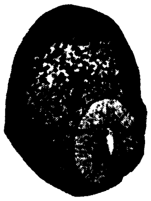
PLATE VI.



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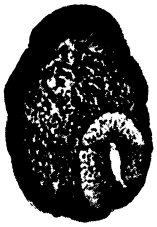

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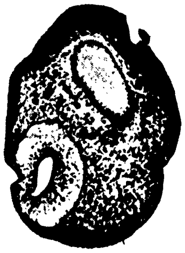

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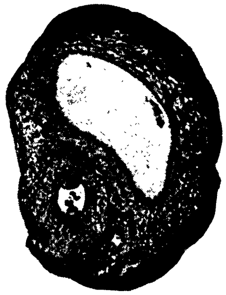

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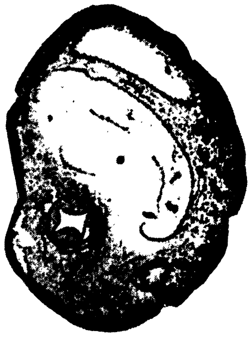

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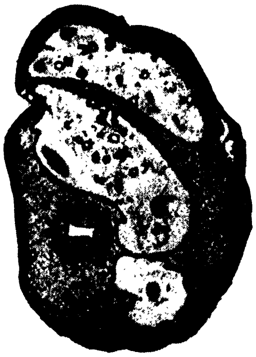

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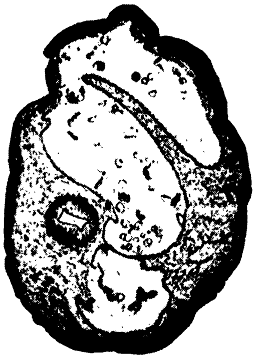

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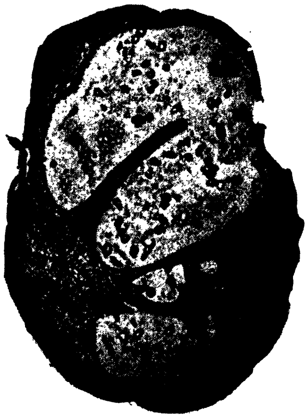

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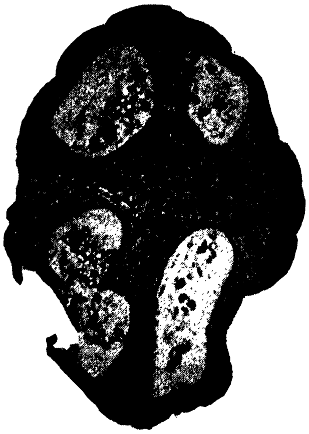

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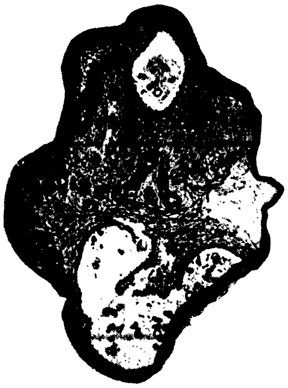
PLATE VII.



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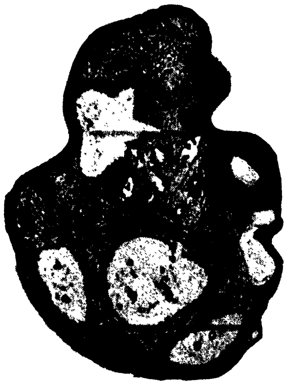

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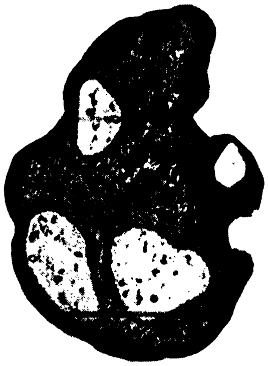

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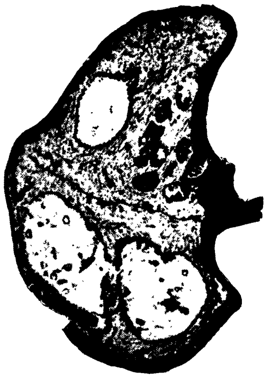

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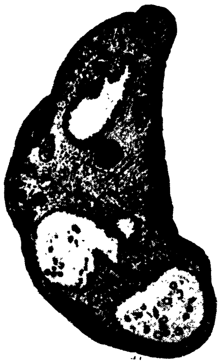

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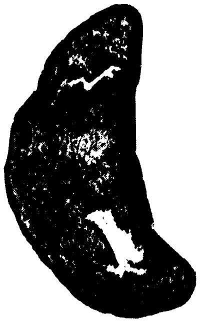

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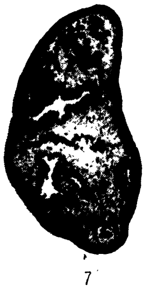

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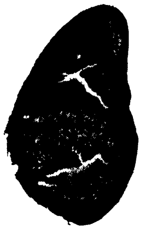

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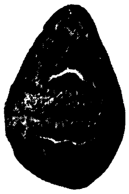

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PLATE VIII.

FIG. 1.

FIG. 2.

PLATE IX.

FIG. 1.

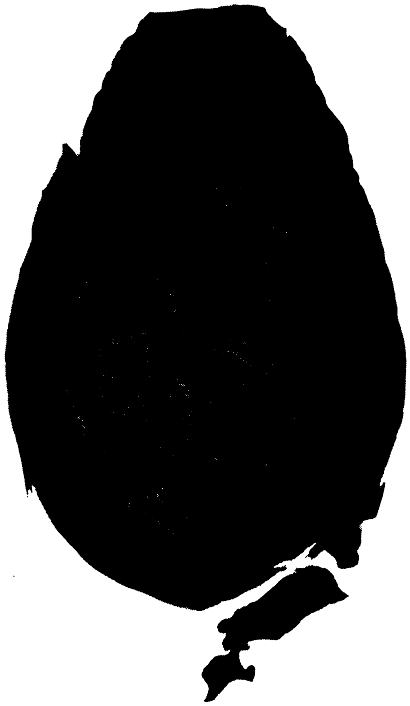

FIG. 2.

PLATE X.

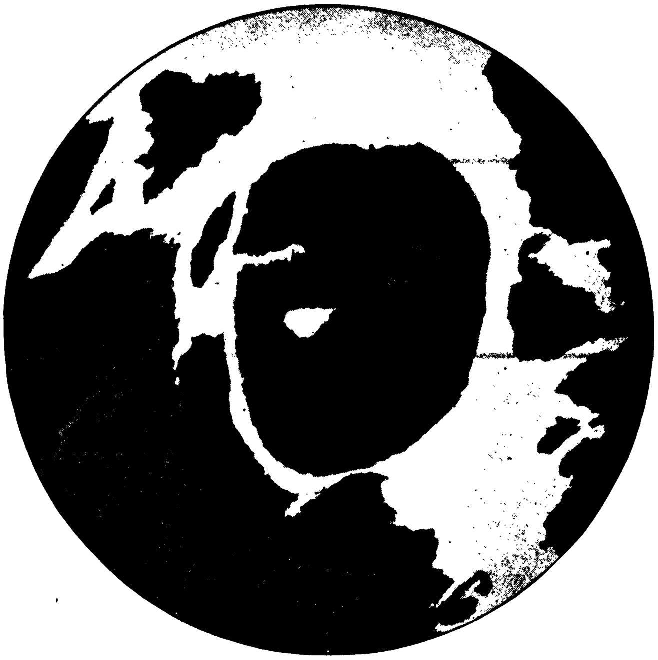

FIG. 1.

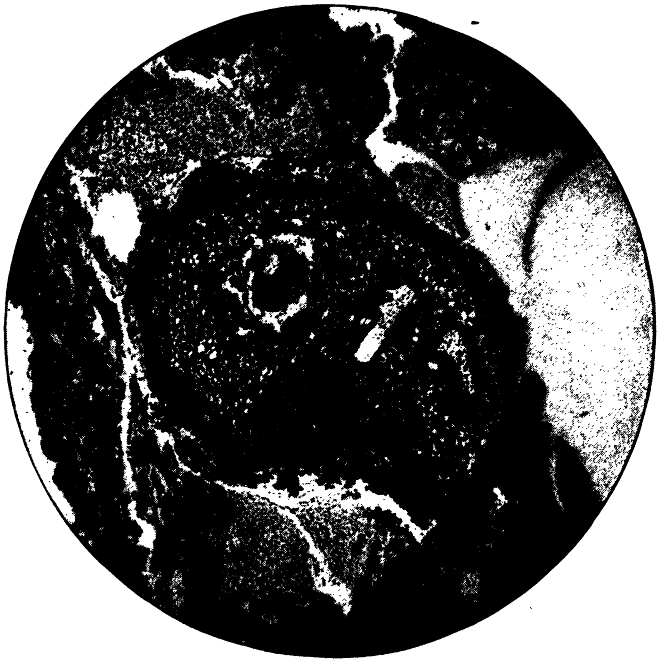

FIG. 2.

PLATE XI.

REVIEWS.

A Compend of Genito-Urinary Diseases and Syphilis, Including Their Surgery and Treatment. By Charles S. Hirsch, M. D. Cloth; 70 illustrations in the text; 1 plate. Pp., xvi + 351. Price, $1.00. Philadelphia: P. Blakiston's Son & Co., 1906.

The abortive treatment of gonorrhœa is given a place in this book, though it does not enter into the modern treatment of the disease.

The metric systm is not used, nor are the metric equivalents of the apothecaries' weights and measures, which latter are used throughout, given. It is much to be desired that all American text-books on scientific subjects either be printed in the metric system or give metric equivalents for other terms used.

In the treatment of the seminal duct and vesicle no mention of the very valuable treatment of chronic affections of these organs by irrigation and drainage of the seminal ducts and vesicles through the vas deferens is made, although Belfield's first paper on this subject was published in the Journal of the American Medical Association in April, 1905.

In the article on circumcision silk sutures are recommended. Silk, as either a suture or ligature material, has scarcely any place in modern surgery and especially not in operations upon parts of the body much exposed to contamination by the excretions.

Under the treatment of the infection of the bladder, vesical amœbiasis is not mentioned.

The statement that suprapubic cystotomy is contraindicated in children is contrary to the best modern surgical practice. The suprapubic method is preferred by surgeons to the perineal operation in children, especially young children, if the calculus is too small or too hard to be easily removed by crushing. The article on suprapubic cystotomy tells us that the bladder must be tightly sutured about the drainage tube for fear of infecting the prevesical space by the escaping urine. This is not necessary. There need be no fear of infection of the prevesical space so long as there is free drainage.

We are told that a floating kidney may be retained in position by a pad placed over the cæcum. Assuming that this treatment is intended for the right kidney we are left in the dark as to where the pad is to be placed in case the left kidney be displaced.

The table of differential diagnosis between epithelioma and chancre states that the microscopical examination of the latter is negative, yet we are told in the foregoing that the *Spirochaeta pallida* is the most probable infecting agent causing the manifestations termed syphylis and that it may be demonstrated in the initial lesion of the disease.

The intramuscular injection of mercury as a means of treatment in syphilis is not given as much prominence as its increasing popularity among syphilographers would seems to warrant. Bichloride of mercury is given first place. It is well known that this is the most irritating of all salts of mercury when so used.

The book contains about 150 typographical errors. An example of some of the most glaring is the use of the term "nephrectomy" for "nephrotomy" under "Cysts of the kidney," page 193.

We never approved of medical compends. They must necessarily omit much that is essential and can not take the place of more thorough reading when they fall into the hands of students, as they usually do. When carelessly printed they are a real evil.

F. W. D.

A Compend of Materia Medica, Therapeutics and Prescription Writing, with Especial Reference to the Physiological Action of Drugs. Based on the eighth revision of the United States Phamacopœia. By Samuel O. L. Potter, M. D., M. R. C. P., Lond. Seventh edition, revised and enlarged. Cloth. Pp., xii + 292. Philadelphia: P. Blakiston's Son & Co., 1906.

The book fulfills the objects of a quiz-compend in that a large amount of information is presented in a systematised and greatly condensed form. Discussions are avoided, and the style is one of short, dogmatic assertion, which is possibly more suitable for a student cramming for examination and memorizing automatically. It is difficult to share the optimism evidenced by the author as to the therapeutic value of certain drugs, and greater discrimination in this regard would increase the accuracy of the book. Thus, the thyroid gland or its extract is described as specific against psoriasis, obesity and certain forms of insanity; anti-diphtheric serum is noted to have benefited typhoid fever, pertussis and asthma.

The printing and binding conform to the rest of the series. The dosage is given according to apothecaries' measure, without metric equivalents.

H. T. M.

The American Illustrated Medical Dictionary. A new and complete dictionary of the terms used in medicine, surgery, dentistry, pharmacy, chemistry and the kindred branches, with their pronunciation, derivation, and definition, including much collateral information of an encyclopedic character. Fourth edition, revised and enlarged. Limp morocco. Pp., 836; 18 illustrations in the text and 29 plates, 28 of which are colored. Price, $4.50. Philadelphia and London: W. B. Saunders Company, 1906.

The present revised edition of this work is, like its predecessors, thoroughly up to date. It furnishes to the student in portable form just what he needs and saves the busy practitioner much valuable time. So far as we have been able to ascertain all the new terms have been included and the semi-encyclopædic character of the work combined with its compactness leaves little to be desired. The author's wide experience both as teacher and physician has enabled him to appreciate what is most needed, and with infinite labor he has condensed the information usually only available in an encyclopedia of medicine into 836 octavo pages. The tables and illustrations are such as are of practical value, and printing and binding are in accordance with the general excellence of the work.

E. C. S.

Stöhr's Histology Arranged upon an Embryological Basis. By Dr. Frederick T. Lewis. From the twelfth German edition by Dr. Philipp Stöhr. Sixth American edition. Cloth; 450 illustrations in the text, 45 of which are colored. Pp., ix + 434. Price, $3.00. Philadelphia: P. Blakiston's Son & Co., 1906.

Dr. Lewis has proved very successful in continuing Dr. Shaper's American edition of Stöhr's Histology.

The arrangement of the book is good. Following a brief but clear description of the structure and vital phenomena of the cell, 134 pages are devoted to general histology and 248 pages to the special systems. A chapter on technique and an excellent index complete the volume. The distinctive feature of the book is the arrangement upon an embryological basis. The description of the adult tissue, or organ, is preceded by a review of the embryogeny, thus enabling the student to gain a comprehensive grasp of the subject and to form a logical conception of the significance of the part studied. The value of the book has been further enhanced by the adoption of the newer anatomical nomenclature. The illustrations, of which there are 450, are in general satisfactory, many presenting early stages in development. It may be suggested that the study of the brain would be simplified by adding a few illustrations at different levels through the medulla, pons, and crura cerebri.

The printing is clear, the size of the volume convenient, and the book can be highly recommended to the student.

H. T. M.

THE PHILIPPINE
JOURNAL OF SCIENCE
B. MEDICAL SCIENCES

VOL II MAY, 1907 No. 2

A CONSIDERATION OF SOME OF THE MODERN THEORIES IN RELATION TO IMMUNITY.

By PAUL C. FREER.[1]

In opening the past annual meetings of the Association it has been customary for the president to call attention to the advances which have been made in matters relating to the medical sciences in the Philippine Islands during the year just completed, and while my purpose is to discuss considerations relating more closely to scientific studies which may serve to increase the efficiency of prophylaxis in the future, it is not out of place briefly to review what has been accomplished since the last reunion of the members of this association.

The outbreak of cholera which was accompanied by the fear that it would once more take on the dimensions of the ones that have swept the Islands in the past, has disappeared; cases of plague are no longer encountered; smallpox is reduced to a minimum and by reason of efficient vaccination any future serious occurrences of this disease need not be feared; improved individual hygiene among a certain proportion of the population has lessened the frequency of dysentery. Hygienically, therefore, we are in a better condition than we were a year ago. The discovery of the prevalence of trematode infections is another advance which eventually will lead to the adoption of measures destined to bring about at least a partial curtailment of the number of persons affected; studies in the habits and breeding places of the mosquitoes, which have been rapidly advanced, will serve as a foundation for plans to combat malaria; and the work done in the study of immunity can not fail to bring with it far-reaching results in the preparation of serums and vaccines. The activity of the veterinary physicians has been such that violent outbreaks of rinderpest, which in certain sections of the Islands threatened a complete destruction of the horned animals, have been prevented by the use of anti-rinderpestic serum, and the methods of preparation of the latter have been markedly improved, so as to increase its efficiency. Work in medical science has also been advanced by the formation of the Army Board for the Study of Tropical Diseases, and by the union of its interests with those of the Bureau of Science. The results of this hearty coöperation are already evident in a series of interesting publications. Work in medical zoölogy has also been undertaken and we expect most fundamental advances in this important branch of tropical medicine during the year to come. The plans for a medical school founded upon modern laboratory instruction and clinics are about completed and we hope, at no distant date, to have an institution which will be as capable as any which may be found in America or Europe of thoroughly training medical students along the lines universally approved to-day.

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[1] The address of the president: Read at the Fourth Annual Meeting of the Philippine Islands Medical Association, Manila, February 27, 1907.

No doubt then, but that the past year has shown a great advance, and no doubt also but that the next one will be equally prolific of results. We can not stand still, for that in all countries and ages has meant retrogression.

In tropical countries medical studies most peculiarly concern themselves with the diseases of infectious origin. The white man coming here runs the liability of contracting certain of them, the native again is exposed to others, and the best means of limiting and combating the outbreaks which occur have been topics for study in all parts of the world. So seriously do America and Europe take the question of limiting infectious diseases that specially endowed institutions have been established for work in methods of prevention and cure. Great masters in this direction of work have arisen, and far-reaching theories as to the causes of natural immunity to certain diseases in individual races and species and as to the production of artificial immunity have been established as the result of difficult experimental work. The advancement of these theories, their discussion, and the laboratory investigations necessary to prove or disprove the various views has served to advance the cause of the study of immunity in the past few years with a rapidity perhaps equaled by no other branch of science, unless it be that of the physical study of the phenomena of radioactivity. It has been the great service of Ehrlich, of Frankfurt, to explain the existing phenomena by a fundamental view of the causes of immunity which is founded upon chemical considerations and which, to-day, is accepted by the majority of workers in this field and, although it has opponents, even the latter have been obliged to bring into their considerations conceptions which, while using different terms, still in many respects conform to the position assumed by Ehrlich.

It is with much diffidence that I approach this subject as a chemist. I had hoped when I selected this topic to have some experimental material to bring before the association, but unfortunately my time has been so occupied in other directions that I have been unable to accomplish what I wished. It will therefore be necessary for you to take such discussion as I have to give, for what it is worth.

A merely casual consideration of the phenomena of immunity to disease develops the fact that we encounter specialized resistance to certain types not only in races, but in families, orders, genera, species and even in individuals. By some processes in the development of various groups of animals, some have become resistant to the change in their condition brought about by the introduction of certain foreign cells, others have become immune to other infections, and so on. This resistance may manifest itself either in a destruction of the introduced cells, in an impairment of their functions, or it may appear as a simple tolerance to their presence without deleterious results. Of course, in this connection we must also consider the cell which is introduced, this too can by ages of development have become resistant or tolerant to the influences of certain of its hosts, and in this way may be capable of resisting destruction in their bodies. The main question to be considered is, How is this destruction or tolerance brought about? In the case of the destruction of an introduced cell, this must either take place by the action of some chemical groups or individuals present in the host, by the aid of which the introduced cell is disintegrated and dissolved, or it must be by physical means, by the action of some form of energy by which chemical changes leading to the disintegration of the cell are inaugurated in the latter. That a proper modification of energy can bring about chemical reaction is too well known for discussion, but it is difficult to conceive how an organism which has been produced while subject to all various manifestations of energy which surround us, should, upon being merely transferred to another organism which has also been produced under the same general conditions, be destroyed by such means only. It is true that in the phenomenon of agglutination we may have, at first sight, some indications of the action of polarity, but here also, to produce the change, certain specific chemical bodies must be present. A *change in energy* undoubtedly takes place during the processes of cell destruction or agglutination and this change in energy must have further, far-reaching results, but as yet the study of immunity has not advanced to a sufficient degree to render a consideration of this phase possible. There remains then as the most probable, *a priori* hypothesis the one which assumes that the examples of cell destruction or agglutination which we observe are brought about by chemical substances present in the host, or by what is still more likely, a combination between certain definite substances in the introduced cell and of others in the host. If now we consider the development of various races from their simplest beginning, we are forced to the conclusion that, in the struggle for existence and in the adaption of individuals to environment, a great variety of substances of gradually increasing complexity must be formed which take part not only in assimilating food but in resisting the encroachments of parasitic organisms. In proportion as, through the ages, the conditions surrounding the race have been altered, as the attacks have multiplied, and the means of resisting them have been applied, there has sprung up the vastly complex chemical system of the higher organisms, and, as the materials which these organisms must assimilate are varied and the means which they must take to resist destruction are also of great variety, it follows that *those chemical bodies which in the host are capable of the destruction of the invader must also be great in number*. It is not possible, considering the specific character of the destruction of certain cells, that one chemical individual should bring about the various types of immunity, indeed, if immunity were due to one chemical individual we would expect it to be universal, and universal immunity would imply the destruction within the body itself of its own tissues, namely, autolysis. Furthermore, the cell which is introduced is in itself resistant in some organisms, non-resistant in others, it may itself therefore have developed immune bodies which must differ from the immune body in the host. We must therefore come to the conclusion that a multitude of chemical groups or individuals must exist in any one organism, which are calculated to resist the invasion of foreign cells. Another question, entirely, is that of the chemical complex or possibly the transferrence of energy which renders the attacking chemical body active. This factor in the destruction of the foreign cell might be uniform throughout, for its action is through the chemical complex which is only one of the destroying factors. If it is simply a method of producing a change of energy it is difficult to conceive why it should act at one time and not at another or, if it is a uniform chemical body, why it should in the sera of certain cases be destroyed at a low temperature and in others at a higher one.

From what has gone before we must conclude that the development of race immunity is a process of heredity, that the substances which confer certain types of immunity on the individuals of a given race have been produced by a course of development concomitant with the other manifestations of evolution and that the chemical bodies which confer the immunity in individuals must be vast in number. In reaching this conclusion we must not lose sight of another means by which immunity can be conferred, namely by the absence of groups capable of uniting with introduced cells or toxins—but this phenomenon also must be the result of heredity.

Exactly the same view applies to conferred immunity. A natural immunity may be increased or one which is scarcely existent may be rendered apparent and protective by the introduction of cells, or the products of those cells. In these cases we produce a more or less specific type which manifests itself either only against the particular cell in question or at least only against those also with which it is closely related. This conferred immunity, in principle, does not differ from the hereditary one; we have only added to the host another set of chemical substances which in themselves were not present before, or at least were present only in small amount. However, bodies related to the introduced substances, or at any rate bodies having similar chemical characteristics must have existed in the host owing to the principles of heredity which have just been discussed and it must be by reason of this similarity or identity that the increased immunity is conferred. The introduced cell then, by reason of its introduction into a host, causes a disturbance of equilibrium in certain cells of the latter, these cells, according to the law by which a chemical system tends to produce a change in the surrounding conditions by which the disturbance can be counteracted, give off or produce chemical substances which tend to destroy or eliminate the invader and in so doing they, responding to the stimulus, go far beyond the point of original equilibrium and so produce the immune substances which find their way into the blood. However, the laws of chemical equilibrium will prevent too great a proliferation of these immune bodies, for there must come a point, if we consider physical laws, when the reaction which produced the immune bodies takes place in the opposite direction, and at this point as many of the immune bodies will be destroyed as created. To speak in the language of chemistry, the reaction must be a reversible one. This condition must arise, or otherwise we could go on in any given case to a condition of infinite immunity. How this reversal is brought about is a matter of indifference. It may be by the formation of anti-immune bodies, it may be by a process destructive to immune bodies which is constantly going on within the cells of the host; in any event the end of the equilibrium must be reached. This new end equilibrium, however, in the case of the conferred immunity, is only an unstable one, the organism must gradually tend to return to its original condition; that is, the conferred immunity must gradually be lost, the rapidity of the change depending upon the nature of the immunity which has been given. Conferred immunity, therefore, differs from natural or hereditary immunity by not being permanent, for in the hereditary type the equilibria have been established through the ages during which the permanent condition has gradually been brought about—the system has adjusted itself to the new equilibrium. It would be interesting to study the production of hereditary immunity in animals, but I fear the task would be a great one, for, as we know, hereditary variations in type are only produced by many generations.

General considerations of the broader phenomena of immunity and some more special analysis of the production of immunity lead us to a chemical view of the question, and we are then brought to the conclusion that there must exist certain types or groups of complex substances which, coming in contact with the introduced cells, cause their destruction. All investigators are agreed that, no matter how these chemical substances have their origin, whether in the conglomerate of cells which go to make up the body, or in certain special ones, such as the leucocytes, once they are free in the circulation they can unite with the introduced cell and can be removed by it. That this is not a phenomenon of adsorption is proved by the fact that there is selective absorption of the immune bodies or of the toxins by a cell, where two or more of the former are present. All investigators also are agreed that, once the immune substance is fixed by the cell, then the additional action of another body or in the case of the toxin, a chemical group therein present, is necessary to cause the final destruction of the foreign organic complex. As to the nature of this last mode of action, opinions differ.

If we come to consider the actual relative mass of a toxin which suffices to cause the death of an individual, or the actual relative mass of a hæmolytic immune body as compared with that of the corpuscles which it destroys, we are forced to the conclusion that the masses of the reactive bodies are extremely small as compared with those of the cells which they destroy. In an immune serum we have water, salts, fibrin, globulin and in short a great proportion of substances which can not be immune bodies, and this fact must be taken into consideration when we regard the actual quantities of these substances present, but, on the other hand, the cell to be destroyed must act as a whole; that is, for example, if the process of destruction is one of hydrolysis, then the hydrolytic action must disintegrate the whole cell. A guinea pig of 300 grams is killed by 0.0025 cubic centimeter or by approximately 10^{-5} times its mass of diphtheria toxin, a horse by 0.3 cubic centimeter of tetanus and a mouse by 1^{10-7} gram; 2.8^{10-6} gram of tarantula can completely dissolve 200,000,000 red blood corpuscles of the rat. It is true that we can not actually determine, in a given case, just what mass of cells is thrown out of function by a given amount of reagent and just how many cells it is necessary profoundly to disturb before serious consequences result to an organism, and in this respect reactions *in vitro* give us little aid, as here we are not acting under normal conditions of life, and we are also ignorant of the relation between the actual weights of reagents employed. These studies are radically different from the ordinary chemical ones, where the stœchiometric relations can accurately be followed.

However, the apparent disproportion between the mass of the reagent which causes the disintegration of the cells and the probable mass of the cells destroyed should draw our attention to the phenomenon of catalysis. The study of catalysis and of catalytic reactions has been advanced most markedly within the past few years, and we have come to have a much clearer conception of the principles underlying the changes brought about by catalytic agents than we formerly had. One fundamental fact must always clearly be borne in mind, and that is *the catalyzer can not change the end equilibrium of a reaction, it can only alter its rate.* A simple example will suffice. If we have a mixture of hydrogen and oxygen, the two gases apparently do not unite at ordinary temperatures, some infinitesimal union however does take place with the formation of water, but the rate of reaction is too slow to be measured and it must be derived by interpolation after a study of the change at higher temperatures. However, if the mixture of gases were to remain for a sufficient length of time, centuries in this instance, a final end equilibrium, in which nearly all had been changed to water and but infinitesimal traces of hydrogen and oxygen would remain, would finally be realized. The introduction of a catalyzer, in this case let us say platinum sponge, will so alter the rate of reaction that it may even proceed with explosive violence, a few seconds sufficing to accomplish that which would take ages under ordinary circumstances. The catalyzer itself, however, is not altered. The recent work of Bredig on the colloidal metals has furnished us with a series of inorganic catalyzers which have the remarkable property of being "poisoned" by chemical reagents similar to those which attack the organic enzymes, and even without such a hint as to a possible resemblance between the inorganic colloids and the organic enzymes, it is quite generally believed that the enzymes are specific organic catalyzers with the power of accelerating the rate of normal reactions which otherwise might be infinitely slow.

The theory of Ehrlich, very briefly stated, is as follows: A cell, having certain chemical groups, is introduced into a body. If, in any of the multitudinous cells of that body there is one which has chemical groups capable of uniting with those of the introduced cell, then, on contact, such union takes place. The attacked cell in the living organism, having its equilibrium disturbed by the occupation of one of its chemical groups, proliferates others which, as I will show further on, may or may not be of the same nature and which are thrown off into the circulation. The proliferated chemical substance must be capable of attaching itself to an albuminous molecule of the introduced cell, for it contains the chemical grouping which assumed the original function of binding the introduced cell. But, in itself, this detached group is not able to bring about the destruction of the foreign invader, it must be rendered capable of so doing by uniting with a chemical substance present in the blood plasma, which is termed the complement. The *immune-body* which is proliferated therefore is an *amboceptor,* with one group it is capable of union with the cell, with the other with the complement, and the latter causes the reaction rapidly to take place.

The immune body (or in the case of toxins the haptophore group) has therefore been termed the "sensitizer" which renders the cell capable of destruction by the enzyme-like complement (or toxophore group), and it has been compared to a mordant. This comparison does not seem to me to be aptly chosen, for the fiber and mordant behave toward each other as two colloids of opposite polarity,[2] the mordant is absorbed by the fiber without apparent chemical union, the fiber itself is not thereby rendered more reactive. The mordant acts independently toward the dye and here also we probably have colloidal absorption. The relations between fiber, mordant and dye are therefore largely governed by physical causes. However, in the case of immune body, complement (or toxophore group) and cell the latter is profoundly altered or destroyed—the destruction appearing closely to resemble the processes of hydrolysis, the immune body apparently serving to bring the enzyme-like complement or toxophore group in closer space relation to the cell, so that one should scarcely speak of either as the sensitizer, but should rather term the complement the *accelerator*. The change somewhat reminds one of the different stereochemical relations present in the formation of lactones from the halogen-substituted fatty acids and some years ago[3] I demonstrated that in these reactions we can have the chemical reactivity influenced by two factors, one taking place through the carbon chain, the other through space. In connection with this view of catalytic action it is interesting to note that Kyes and Sachs[4] in studying the acceleration of the hæmolytic action of cobra venom by lecithin have shown that the more snake venom is used, the less lecithin is necessary to effect complete hæmolysis and *vice versa,* the more lecithin, the less venom need be used for the minimal completely solvent dose.

It will be seen from the above that the proliferation of immune bodies according to this view is brought about by a disturbance of equilibrium within the cell, that therefore the production of immune bodies is a *normal process* accelerated by the introduction of the foreign cell. Under undisturbed conditions of equilibrium these *or similar* immune bodies are also most certainly being given off, but owing to their gradual proliferation, they undoubtedly disappear without causing a change in the normal equilibrium. By the introduction of the foreign cell, however, the *rate of the reaction* is altered so as to throw a large amount of these immune bodies into the circulation at one time. This production of immune bodies, therefore, has much the appearance of catalysis, where the catalyzer which proliferates the immune body is generated within the body cell itself.

[2] W. Biltz: *Ber. d. chem. Ges.* (1904) **37**, 1766.
[3] *Ann. Chem.* (Liebig) (1901) **319**, 345.
[4] *Berl. Klin. Wchnsch.* (1903), **40**, 21.

I have mentioned above that, under undisturbed conditions of equilibrium the immune bodies or *similar substances* are undoubtedly constantly being given off. I had in mind some of the phenomena of organic chemistry in which the change of one stereochemical form into another is brought about, or perhaps more aptly, the alterations which we observe from the keto- to the enol-form and *vice versa*. We have bodies, for example, which when prepared under certain circumstances, react like ketones or aldehydes, these same bodies may spontaneously or under slight stimulus, become altered to unsaturated alcohols and again under others they may at times appear as ketones and at others as alcohols. The same condition may apply when the equilibrium of the living body cell is disturbed by the introduction of the foreign one—the immune bodies which are normally proliferated may be changed in this manner in their chemical structure by the introduced cell so that now they become capable of independent existence and possibly also of firmer union with other cells and so that they are also able to fix the complement. This view does not seem improbable when we consider that we have immune bodies of apparently similar origin, some of which are capable of firm fixation to the introduced cell, others of which are but loosely bound and still others which have their avidity increased by union with the complement.

While I have shown above that the immune bodies may be produced by catalysis of normal processes, it does not follow that they are themselves catalyzers when they unite with the introduced cell, with the toxin, or in the case of toxins themselves, with the body cell, for it has conclusively been shown that such union does take place and that then these bodies are either removed from the serum or that they unite with the toxin. But, if the view of catalysis is correct, them these bodies, when so attached should, at a very slow rate of reaction, accomplish that which they do rapidly under the influence of the complement, for the catalyzer simply *accelerates* an otherwise normal reaction; in other words, to select an example from the phenomena of hæmolysis, the lysis of a red blood cell to which an immune body is attached, should take place very slowly as a normal reaction, which would proceed very rapidly to an end equilibrium by the addition of the complement. Individual test-tube experiments in hæmolysis have not extended over a sufficient period of time to prove or disprove this view, and it would be interesting to follow out this subject if the experimental difficulties are not too great.

On the other hand the complement which is attached to the amboceptor, either permanently, as in the case of the toxins, or which is taken up from the blood serum, as with the hæmolysins, seems much more closely to resemble the catalyzers, but if it is a catalytic agent it must accelerate a reaction which otherwise would take place with extreme slowness, with such slowness, indeed, that without such a catalyzer no noticeable difference of the normal equilibrium would take place. Dr. Strong, acting on this view, has conducted a series of experiments with diphtheria toxin in guinea pigs which were simultaneously injected with minimal doses of pure hydrocyanic acid, acting on the theory that this substance, which so markedly "poisoned" Bredig's colloidal inorganic catalyzers, would do the same with the organic ones, and that hence the action of the diphtheria toxin should be delayed, with a gradual recovery of virulence as the hydrocyanic acid disappeared. The results were entirely negative. However, this one series proves nothing excepting that other means of experimentation must be resorted to. The work could probably be more successfully carried out *in vitro.*

The process of cell destruction by aid of the complements which are present in the serum has one interesting phase which causes it to differ from that where the catalytic agent, if I may use the term, is a chemical individual which can be isolated, such as lecithin. In the case of the serum complement there is a time of incubation—that is, the reaction proceeds with an increment of increase in unit time—which can only be brought about by the relatively increasing amount of catalyzer present, in other words, the complement must either be *formed during the reaction* or it must be liberated as soon as its work is done, but in the latter event, *in vitro;* we must also have a steadily diminishing amount of substance in unit volume to be acted upon, and a consequent gradual retardation. The study of the *rate* of these reactions and the resulting curves is therefore of fundamental importance, and in the case of hæmolysis the experimental difficulties would not seem to be too great to overcome. It would certainly be well to determine the proportion of complement and immune body as compared with that originally present remaining in hæmolyzed blood after the reaction is complete, although in this connection care must be exercised not to confuse the possible effects of substances like lecithin, which could be separated from the stroma, with the true serum complements.

In closing I wish to call attention to one phase of the researches on immunity which would repay further investigation. Ehrlich has said:

> I accept the existence of haptophore groups exclusively in compounds such as the food stuffs which can enter into the composition of the protoplasm or which, such as the great series of poisonous or not poisonous products of the metabolic changes of living cells, can enter into a combination similar to that of the food stuffs.
>
> The deep-seated difference between the alkaloids, glucosides or medicinal agents of known chemical constitution and those substances which possess haptophore groups and which are therefore capable of liberating antibodies during the process of immunization is shown by the fact that none of the former class have ever been able to give rise to any antibody production of consequence.

However, I believe that this seemingly impassible gulf can be crossed. Undoubtedly, we can place on one side poisons from which no trace of an immunity reaction can be secured and on the other substances, the products of life action, which give all the typical phenomena which we are accustomed to associate with what we term immunity, but surely the line can not be an abrupt one and hence, in this investigation we must seek chemical individuals of known constitution which, under proper conditions, are capable as synthetic bodies of entering into metabolic reactions. The recent work of Emil Fischer on the amino-acids and the synthetic formation of the polypeptids is suggestive and it is with similar synthetic substances that we may hope to see our next great advance in the study of immunity.

The problems to be encountered in immunization are difficult, they involve painstaking experimental work and close reasoning and thought but, as I have endeavored to show, the modern views of chemistry and physics are all on the side of the worker in immunity; he has but to reason closely to untangle one more skein of the web, and his reward is great. In place of the constant fear of recurring serious epidemics of devastating diseases with their accompanying vast expenses, and in place of the constant vigilance necessary to prevent serious outbreaks of infectious diseases, the worker in immunity may possibly as a result of his studies in the future be able to render a community practically safe from all but negligible sporadic cases. We could then dispense with the rigors of quarantine and its interruption of commerce, or with the enormous loss of life consequent upon the occurrence of epidemics. True, the prejudice against methods of immunization is as yet great, much more of a scientific nature needs to be done, much of a missionary character undertaken, but then not many years ago the very fact of the causation of disease by microörganisms was the subject of the bitterest dispute and many members of the medical profession itself were sceptical as to the results which were to follow. However, the opposition of the profession has practically disappeared, that of the laity will of necessity follow, and our successors will find the way for future advance cleared for them by the pioneers of to-day.

ON THE CULTIVATION OF A BOVINE PIROPLASMA: A PRELIMINARY COMMUNICATION.

By M. Miyajima.

(From the Imperial Institute for the Research of Infectious Diseases, Tokyo, Japan: Director, Prof. S. Kitasato.)[1]

One of the most interesting subjects in medical science, especially in tropical medicine, is an investigation of the parasitic protozoa. So many valuable discoveries have been made in the study of these organisms that it is not necessary here to mention them, but nevertheless at present the knowledge of protozoölogy is not so far advanced as that of bacteriology. This condition may be ascribed to the fact that on the one hand, the life history of the class protozoa is most complicated and on the other, the protozoan parasites can not be cultivated so easily as those of bacterial origin. However, the efforts of several workers have made the study of the pathogenic protozoa more accessible. The mode of transmission of *Piroplasma bigeminum* was clearly demonstrated by Smith and Kilborne;[2] later the relation of mosquitoes to the malarial parasites was discovered. The pure culture of the pathogenic protozoa outside of the living body was first accomplished by Novy and McNeal[3] in the case of *Trypanosoma lewisi.* These fundamental studies have thrown much light upon the methods of investigation in modern protozoölogy. It is my belief that we owe so much to your countrymen in the progress of this science that I have mentioned their admirable investigations in this field before entering upon the discussion of my own subject.

Among the problems in modern protozoölogy which have greatly attracted the attention of scientific men, that one connected with the view of the late Professor Schaudinn[4] with respect to the relation of the hämocytozoa to trypanosomata may be mentioned. Schaudinn announced that the hämocytozoan known as *Halteridium* develops into a trypanosoma in the body of the mosquito; furthermore, he believes that piroplasma may similarly assume a trypanosoma form in the course of its development. However, Novy and McNeal[5] reached a different conclusion from the above observer, for they affirmed definitely that the trypanosomata of birds are not related entirely to hämocytozoa such as *Halteridium* and *Leucocytozoa*.

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[1] Read at the Fourth Annual Meeting of the Philippine Islands Medical Association, March 1, 1907.

[2] *Bull. Bureau of An. Industry,* U. S. Dept. of Agr. (1899).

[3] Novy and McNeal: Contrib. to Med. Research (1903), 549.

[4] *Arb. a. d. k. Gsndhtsamte* (1903–04) **20,** 387.

Again, Rogers[6] succeeded in securing a culture of the small, intracellular parasites of *Kala-azar* known as the Leishman-Donovan bodies, in which the organisms developed into large, motile flagellates, most closely resembling young trypanosomata which had not yet developed an undulating membrane.

Thus, our problem on the relation of hämocytozoa to trypanosomata becomes more complicated and must be settled by further observations. For this reason I took as my material for this investigation a form of bovine piroplasma which is readily accessible in Japan, and made some observations in coöperation with my assistant Dr. Irikura.

Observation 1.—In a paper[7] "On the Piroplasma Found in Japanese Cattle," I have, with Dr. Shibayama, shown that a large percentage of Japanese cattle is infected with a species of piroplasma which seems to be similar to the parasite of coast fever (*Piroplasma parvum*). In the course of an extended study we were able fairly often to demonstrate the same parasites in the blood of the native cattle of Korea. These parasites, found in apparently healthy animals, have been mostly of the small bacillary type, the large pyriform or ring-shaped bodies only being seen occasionally. R. Koch[8] in one of his latest publications, described a peculiar cross-form which distinguishes *Piroplasma parvum* from its allied parasites. After a prolonged search this form was also encountered in the blood smears prepared from our native cattle, although only in small numbers (Pl. I, fig. 1). However, we have not as yet observed any symptoms characteristic of coast fever in our infected Japanese cattle, although the parasites found therein might morphologically be identical with *Piroplasma parvum* Theiler. There are diverse views in regard to the pyriform body of this species. Some observers believe that the pyriform body occasionaly met with in the blood of an animal suffering from coast fever rather represents a mixed infection with "Texas fever," while others consider it to be a distinct form occurring in the life cycle of *Piroplasma parvum,* as is the case in the other varieties of this group. In support of the latter view it may be stated that we have observed the large pyriform body occurring regularly, although in small numbers, in the circulating blood of every infected animal, and in Japan an infection with Texas fever is out of the question.

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[5] *Am. Med.* (1904), **8**, 932.

[6] *Quart. J. Micr. Sc.* Lond. (1905), **48**, 367.

[7] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1906) **54**, 189.

[8] *Deutsche med. Wchnsch.* (1905), **47**, 1865.

As is already known, the parasite *Piroplasma parvum* is also characterized by the fact that the infection can not be transferred by the inoculation of the blood itself, and in this respect we have observed that it differs greatly from its allied organisms, *P. bigeminum, P. carus,* etc.; however, on the other it shows a great similarity to "Hæmoprotens" which is a common parasite in the blood of birds.

Observation 2.—Since the discovery of the important rôle taken by ticks in infections with piroplasmata, many observers have in vain endeavored to prove the existence of the developmental forms in the bodies of the ticks which live on infected animals. Very recently an actual demonstration of a developmental change of the piroplasma was given for the first time by R. Koch.[9] He described certain developmental forms of *Piroplasma bigeminum* and *P. parvum,* the final stages of which are as yet unknown. After his work was published, we endeavored to repeat his experiments by feeding the cattle tick *Rhipicephalus australis,* which is found abundantly on the native cattle of Japan and is regarded as being a probable carrier of *Piroplasma parvum.* After several unsuccessful trials, my efforts were directed toward the cultural method by which Rogers and other observers were able successfully to demonstrate the flagellate stage of the Leishman-Donovan bodies.

The following different culture media were tested in our preliminary experiments on the cultivation of the piroplasma; blood agar, sodium citrate, both acidulated and nonacidulated; beef extract, peptone water; calf's serum; physiologic salt solution; common bouillon, etc. On the 4th of July, 1906, we first observed a few motile organisms in one test tube which contained a small amount of infected blood mixed with acidulated sodium citrate solution, prepared according to the method of Rogers. The entire series of cultures was then carefully examined but no evidence of development of any motile organism was obtained except in the bouillon ones. This medium was apparently the most favorable, large flagellates having abundantly developed therein after four days' incubation at a room temperature. Only a slight multiplication of the organisms took place in the first culture which we obtained in acidulated sodium citrate.

The length of the organism at its full-grown stage, as it is observed in cultures, is about five times the diameter of an erythrocyte. It, at this time, possesses a well-defined undulating membrane and a long flagellum. The position of a nucleus and blepharoplast in the body of the flagellate renders it impossible to distinguish it from a typical trypanosoma developed in a culture. (Pl. III, fig. 2.)

The method which we have employed is a simple one, and is practically the same as that used by Rogers.[10] The blood containing intracellular parasites, is drawn from the jugular vein and then quickly defibrinated under strict precautions so as to avoid bacterial contamination; it is then directly mixed with ordinary nutrient bouillon in proportions varying from one-fifth to one-tenth, and placed aseptically in sterile test-tubes which thereafter are maintained at a temperature of 20° to 30° C. The development of the parasites in a successful culture takes place in the following manner: On the first day no motile form is seen; on the second, there can be observed a certain number of peculiar cells which occupy the upper layer of sedimented corpuscles and which macroscopically appear as a series of whitish dots. Very few motile forms resembling typical trypanosomata are visible in these cells on the third day after incubation, but thereafter the trypanosomata multiply vigorously and reach the maximum number between the tenth and fourteenth day. (Pl. III, fig. 12.)

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[9] *Ibid* (1905) **47**, 1865, and *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1906) **54**, 1.

In a culture kept at room temperature, the trypanosomata remain motile until forty-five days later, at this time most of them have undergone degeneration and globular cells with irregular granulations result. In a culture preserved at a lower temperature, ranging from 10° to 20° C., the organism on the contrary remains alive until three months after the maximum number has been reached. It is noteworthy that subcultures are also readily obtained by inoculating from the original strain into a new blood bouillon, as in the case of *Trypanosoma lewisi.*

The most important factor in securing the multiplication of the parasites essentially consists in great precautions in avoiding the slightest contamination with bacteria, as is the case with other cultures of protozoa.

We have already made miscroscopical examinations of the blood in over 200 cattle and among them not one has proved to be infected with trypanosomata; moreover, all varieties of bovine trypanosomiasis are entirely unknown in our country, at least up to the present time no one has demonstrated a case of such infection.

As is well known, there are many protozoan parasites which infect a living host without the latter manifesting any pathological symptoms; it is also a fact that microscopic examination is not as delicate a means for the detection of a small number of parasites as is the cultural method, which is essentially used in the study of bacteria. From a consideration of these conditions it seems more natural at once to consider the trypanosoma-like flagellates found in our cultures to be true trypanosomata, which, owing to their extremely small number in the original blood, would fail of detection by means of the microscopical examination alone.

However, the relation between piroplasma and trypanosoma, although supported by the views of Schaudinn and other observers, is still a question of much interest and one which needs further investigation and therefore our observations on the parasites were extended further. The results of this study are here presented in as brief a manner as possible.

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[10] *Lancet* (1905) 1, 1684.

Observation 3.—Twenty-one native cattle were carefully examined both by microscopic and cultural methods at the same time. Of this number, nine were shown by microscopic examination to be infected with *Piroplasma parvum;* in only seven of these cases were the flagellate organisms found in the cultures; in the remaining two, the cultures gave no growth of flagellates. Microscopic tests failed to detect any parasite in the other twelve cases and, similarly, the cultural method gave a negative result. Therefore, the almost constant occurrence of piroplasma in the blood and of flagellates in the culture renders the existence of certain relations between them very probable and we can hardly regard this occurrence as a merely accidental phenomenon.

I have also ascertained the minimum doses of infected blood which would give a growth of the flagellates in cultures. For this purpose a freshly prepared culture medium was used, which consisted of the blood of an uninfected calf or rabbit and common bouillon. Each tube of the blood-bouillon was inoculated with a different amount of the infected blood which harbored a fair number of intraglobular parasites. I have obtained some interesting results from a series of experiments along this line, namely, that the quantity of the incubated material is a matter of indifference in respect to the development of trypanosomata in the cultures, since one platinum-loop full of the blood gave exactly the same result as if one cubic centimeter of the same material was used. In other words, the germ which gives rise to trypanosomata in the culture exists even in so small an amount of the blood as one platinum loop-full, or one drop. These facts suggested a possible means of detecting the flagellates by direct blood examination, if they exist originally in the blood of an infected calf. However, a large number of blood preparations, both fresh and stained, were thoroughly investigated, but no flagellates were observed. This mode of observation was *repeated* several times, but every series gave negative results. Furthermore, we have centrifugated a mixture of the infected blood and salt-solution at a low temperature, to prevent a possible development of the parasites. The sediment was than removed drop by drop from the upper layer of the centrifugated material and subjected to careful microscopic examination. It was expected by this procedure to obtain direct evidence of the existence of trypanosomata in the blood, but our trials failed to detect any motile or flagellated organism.

Observation 4.—The most effective evidence in favor of the view that the flagellates originate in the culture may be secured by a morphologic study of each developmental stage of the parasites, but many difficulties are encountered in such investigations. On the one hand the scanty number of the pyriform bodies in the material treated renders the work most difficult and on the other, when a flagellate once starts to develop in the culture, it multiplies so quickly as very much to interfere with the work of tracing each developmental stage. After extensive researches, certain interesting forms of the parasites were found in the stained films prepared from young cultures, which afterwards gave a growth of numerous trypanosomata. Soon after the culture was made, a few round cells, which morphologically were identical with the free pyriform bodies, were seen in stained preparations. (Pl. I, fig. 2.) Within the first twenty-four hours after incubation, the diameter of the round cells (Pl. I, fig. 3) increased, and it finally became twice that of an erythrocyte. Associated with the enlarged cells there also were irregular amœboid forms (Pl. I, fig. 4) similar to the former in nature. In this stage the chromatin of the cells was distributed more or less irregularly in the cytoplasm; in some it was diffused throughout, while in others, distinct chromatin granules were demonstrable. A further advanced stage of the parasites which occurred in cultures was a vacuolated, globular form which retained its staining property just as nonvacuolated cells do. (Pl. II, fig. 5.) As a result of the distension of the vacuole, the parasite gradually assumed the shape of a large ring, of which the thickest part contained some chromatic dust. (Pl. II, fig. 6.) In addition to these, there were many degenerated cells of an allied nature, which were principally characterized by the numerous vacuoles and hypertrophic chromatin. (Pl. II, fig. 7.) The change which took place within the next twenty-four hours was very interesting. The ring-shaped cells (Pl. II, fig. 8) in which the chromatic dust had already become rearranged so as to form a large nucleus and a small blepharoblast, transformed themselves into spindle-shaped organisms in which no visible flagella were present. Associated with this form was observed the flagellated motile parasite (Pl. III, fig. 9) which morphologically was almost identical with the former.

Finally, in the 72-hour culture we obtained a flagellated form showing the development of an undulating membrane. The size of this form, which was that of a typical trypanosoma, increased and afterwards it multiplied in the manner usual for true trypanosomata—that is, by longitudinal fission (Pl. III, figs. 10 and 11)—thereafter, as has already been mentioned, the fullgrown and divisional forms increased in number.[11]

The recent observation of R. Koch[12] in regard to the developmental changes of piroplasma are of great interest, for he described several forms encountered in the body of ticks which in some respects resemble those obtained by us in culture. In the first place, his most predominating forms are peculiar cells, provided with numerous fine, protoplasmic processes; furthermore, he described a large, rounded form and a club-shaped parasite which closely resembled the oökinetes of *Plasmodium* and *Halteridium*. These important observations were later confirmed by Kleine[13] who used a different method of investigation. He secured a culture of canine piroplasma by diluting the infected blood with salt solution and was also able to demonstrate the peculiar, star-shaped cells and globular forms in his cultures. The further developmental forms, after the majority of the cells had undergone degenerative change, did not appear in cultures of *Piroplasma canis.*

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[11] A detailed account of these developmental forms of *Piroplasma parvum* must be left for our more complete paper on the subject.

[12] *Deutsche Med. Wchnsch.* (1905), 47, 1865.

In comparing the results obtained by both of these authors with our own, we find many points of resemblance in respect to the morphological features of the parasites. The star-shaped forms described by Koch and Kleine probably correspond to the amœboid cells (Pl. I, fig. 4) found in the early developmental stage of our parasite, though the latter does not possess such a fine plasmic process as the former. The globular cells (Pl. I, fig. 3; Pl. II, figs. 5 and 6) are a common form occurring in the development of all cases, and the club-shaped ones figured by Koch seem to correspond to the first stage of the motile flagellate observed with our parasite. On the other hand, the successful culture of the intracellular parasite was first accomplished by Rogers with the Leishman-Donovan bodies. The facts demonstrated by the above-mentioned authors therefore greatly support our own observations. It may be well here to add that the mode of formation of a flagellated form in the case of our parasite is apparently different from that of others; especially does it differ from that of the *Leishmania donovani,* which transforms to the flagellate by direct elongation of an enlarged globular form, whereas the piroplasma, according to our observations, develops in the unusual manner described above.

Observation 5.—The final means by which we are able to reach a definite conclusion in respect to the trypanosomata consists of animal experiments with the cultures. It is a well-known fact that with the parasite of coast fever (*Piroplasma parvum*) the direct inoculation of the blood into cattle, even in a large amount, always fails to give rise to infection with the appearance of parasites in the animal so inoculated, and as regards this point the parasites investigated by us were already proved to be identical with *Piroplasma parvum.* We selected three calves which, after repeated examinations by microscopic and cultural methods, were demonstrated to be free from parasites. The animals were then inoculated with a culture containing motile trypanosomata in abundance and as a necessary prerequisite to the successful completion of the experiment, they were kept under such conditions that danger of infection from any source, especially from ticks, was avoided. The blood of the calves, after the injection, was microscopically and culturally examined from time to time. One of the animals proved itself to be refractive, as is observed in the case of the direct inoculation of the infected blood. However, the others were infected with parasites, which fact was first proved by the cultural method and later demonstrated to be the case microscopically. In one instance, eight days after inoculation, the blood of the susceptible animal began to give a growth of the flagellated parasite in culture, whereas seventeen days later by the aid of the microscope alone, the intracellular parasites were visible in the same animal. (Pl. III, fig. 12.) The number of our experiments on animals is not as yet sufficiently large for us definitely to give the duration of incubation of the infection and other details.

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[13] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1906), **54**, 10.

The foregoing discussion brings us to the following conclusions:

1. A variety of hämocytozoa known as *Piroplasma parvum* can readily be cultivated outside of the living body.

2. The parasites undergo the developmental change in blood-bouillon and finally take the form of a typical trypanosoma. This trypanosoma can not be detected in the blood of infected animals.

3. A simple mixture of blood and bouillon is the most suitable medium for the cultivation of protozoa such as *Piroplasma parvum* and *Trypanosoma lewisi.*

ILLUSTRATIONS.

[Photomicrographs by Mr. Charles Martin, photographer of Bureau of Science, Manila.]

PLATE I.

FIG. 1. Blood smear from a calf inoculated with a culture containing many trypanosomata; a typical cross-shaped form and many intraglobular parasites. Mag about 1050×1.

2. Swelling of intracellular parasite after the blood is drawn from a naturally infected calf. Mag. 1570×1.

3. Extracellular globular parasite in a three hours' culture at 25° C. Mag. 1570.

4. Amœboid parasite in a three hours' culture at 25° to 30° C. Mag. 1580.

PLATE II.

FIG. 5. Large, globular parasite with two small vacuoles developed in twenty hours' culture at 25° to 30° C. Mag. 1570.

6. Large globular forms with one large vacuole developed in twenty hours' culture at 25° C. Mag. 1570.

7. Irregular, large, vacuolated forms occurring in forty-eight hours' culture at 25° C. Mag. 1100.

8. More advanced stage in the development of the globular parasite. Body crescentic. Forty-eight hours' culture at 25° C. Mag. 1305.

PLATE III.

FIG. 9. Flagellated form occurring in a forty-eight hours' culture at 25° to 30° C. Mag. 1570.

10. Enlarged flagellate possessing two blepharoplasts and flagella with but a single nucleus; preliminary divisional form occurring in three days' culture at 25°. Mag. 1305.

11. Smear from a four days' culture grown at room temperature (20° to 27° C.), showing (*a*) typical, dividing trypanosoma; (*b*) a slender flagellated form. Mag. 1305.

12. Smear from seven days' culture incubated at room temperature (20° to 27° C.) Showing numerous trypanosomata. Mag. 820.

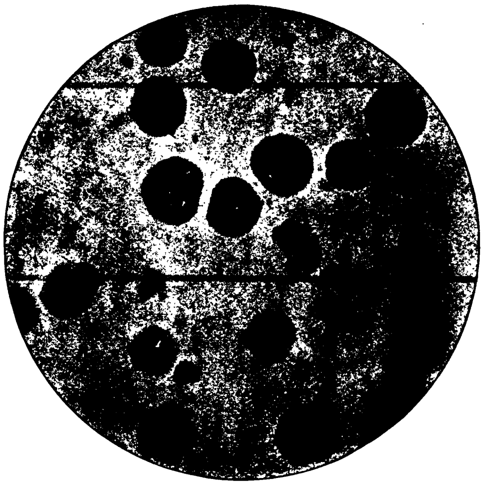
FIG. 1.

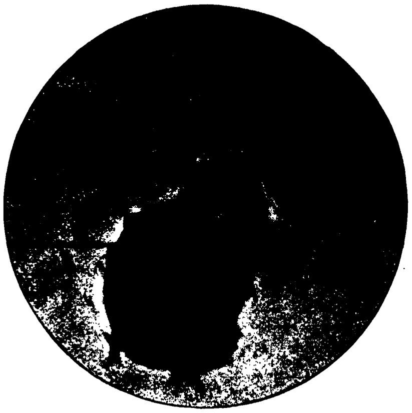
FIG. 2.

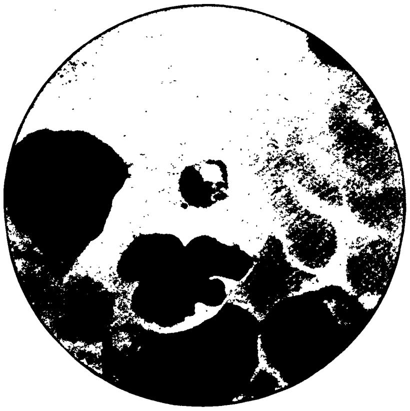
FIG. 3.

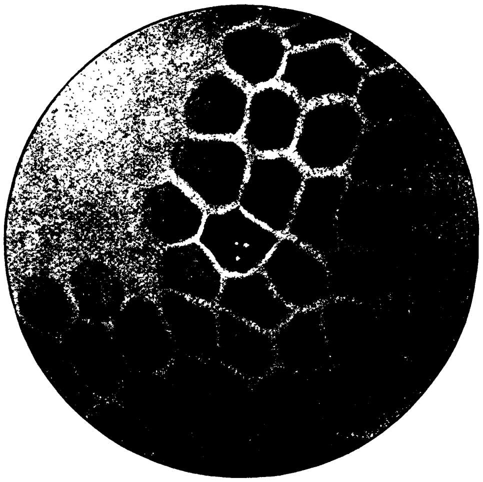
FIG. 4.

PLATE I.

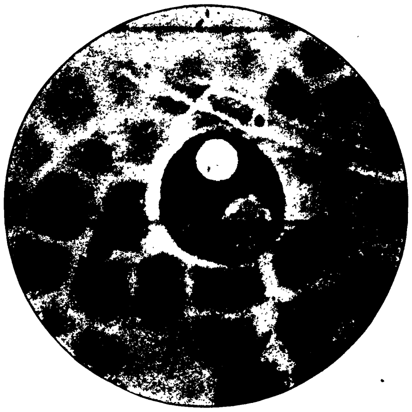

FIG. 5.

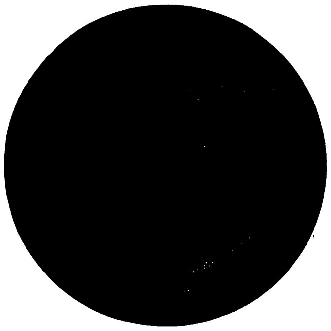

FIG. 6.

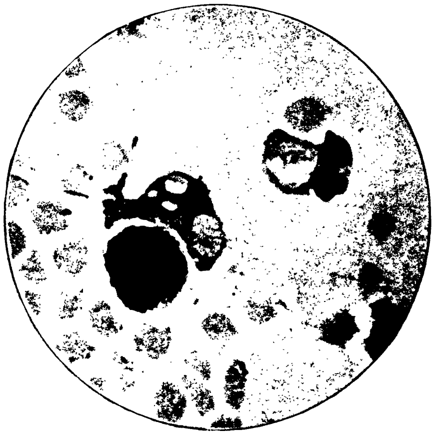

FIG. 7.

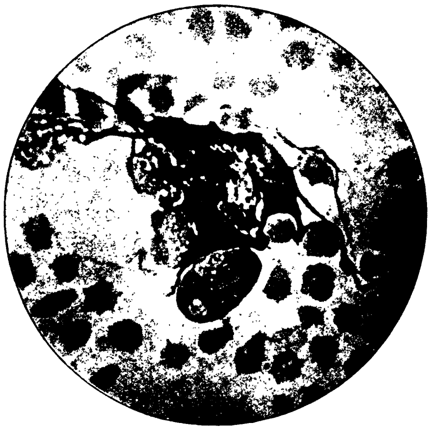

FIG. 8.

PLATE II.

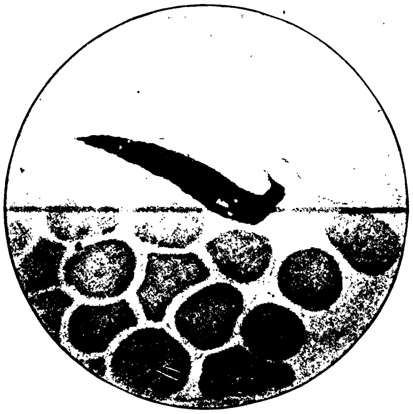

FIG. 9.

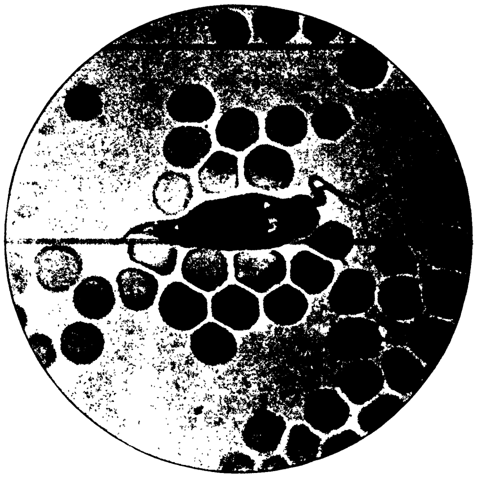

FIG. 10.

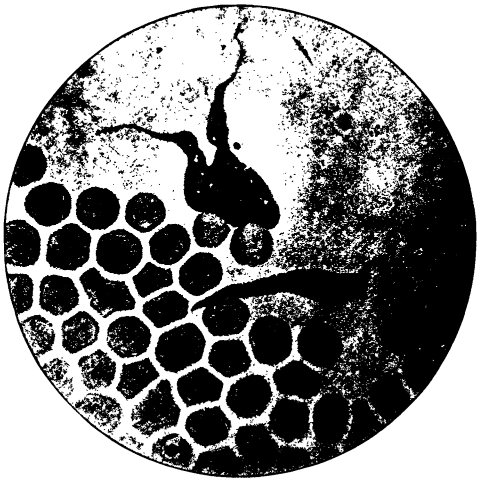

FIG. 11.

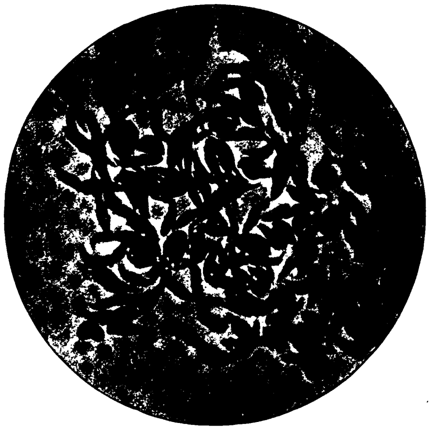

FIG. 12.

PLATE III.

EXPERIMENTAL INVESTIGATIONS REGARDING THE ETIOLOGY OF DENGUE FEVER, WITH A GENERAL CONSIDERATION OF THE DISEASE.[1]

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[1] Read by abstract at the Fourth Annual Meeting of the Philippine Islands Medical Association, March 1, 1907.

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I. INTRODUCTION.

Having been instructed by the Surgeon-General of the Army to investigate the cause of dengue and to determine the possibility of the transmission of the disease by mosquitoes, we undertook the experiments to be detailed below when the opportunity was afforded us by the occurrence of the disease in epidemic form at Fort William McKinley, Province of Rizal, Luzon, P. I., 5 miles out of Manila.

The incidental investigation of available literature on the subject indicated to our minds that a brief discussion of all the features and phases of the disease would not be out of place, as there are discrepancies to be reconciled and certain doubts to be settled, some of which may possibly be ended by our conclusions, while on the other hand some points on which our experiments leave us in doubt seem to be strengthened by the history of the disease as recorded in the literature.

The literature has not been nearly as accessible to us as we could wish, and several articles, both old and new, which we desired to consult, were not available.

The first question that merits discussion in the consideration of this disease concerns its existence as a disease entity. At times the question of identity with influenza has been raised.

However, in our opinion, the study of the writings of those men who have had experience with dengue should promptly and finally settle the question. The agreement in the descriptions from 1827 and 1828, when Dickson, Squaer, Osgood, Dumaresq and others wrote of the disease, down to the latest articles accessible, such as those of Guiteras and Cartaya, is remarkable, while the almost simultaneous appearance from opposite sides of the world of two reports agreeing so closely throughout as do those of the Australian committee and of Guiteras and Cartaya, is even more striking, and the clinical picture drawn from these writings from almost a century of literature and from every part of the tropical and subtropical world is quite distinct and unique. It has very little in common with the picture of influenza, and many points of difference, and in our opinion it would be only the exceptional case that would give rise to confusion.

II. EPIDEMIOLOGY.

1. HISTORY.

The written history of dengue extends back clearly as far as 1827, while it is quite probable that it was recognized and described much earlier: By Rush (1), in 1780, as "bilious remittent fever;" by Boylon, in Java, in 1779, while Thomas (3), speaks of Pazzio describing an epidemic in Seville, in 1764–68, which was traced to Africa. Dickson's (2) writings, however, first brought the question into prominence, at least with Americans, and his observations and descriptions are applicable to the disease as it occurs to-day.

The number of writers, from the earliest down to the present, who have likened the disease, in its causation and spread, to yellow fever, is very striking.

Foster says that most of them have considered it a modified yellow fever. We do not so infer from such literature as we have seen. They speak of it as having a common cause, but as these statements were made before the specific causes of any of the fevers were known, we take it that the writers meant merely that the cause of both was a "miasm," an atmospheric condition, the state of the ground water, etc. We have not found any author who states that the one disease may arise from the other.

The disease has occurred in most widespread epidemics in the tropical and subtropical world, not only covering large areas of country, but attacking a greater proportion of the inhabitants than probably any other known disease, and usually also affecting most of the other members of a family after one has been attacked. That being the case, it is rather surprising that a belief in its contagiousness has not been practically universal.

As a matter of fact, only a minority of the authorities whose writings are accessible to us have expressed an unqualified belief in its contagiousness, while many have rejected it entirely.

Dickson considered it very highly contagious, while many of his contemporaries liken it to yellow fever in its manner of progression. Most writers express themselves as in doubt on the subject. Wragg denied contagiousness, as did Horlbeck (4).

Holliday (6) wrote to sixty practitioners who had large experience with the disease, asking their opinions as to its contagiousness. Forty-five replied that it was simply epidemic, four that it was contagious, two were in doubt, and the rest did not answer.

One point on which the writers, from first to last, almost unanimously agree is the influence of atmospheric conditions. Hot, sultry weather, with abundant rains, is by all thought to favor the occurrence of epidemics. Nearly all also agree in stating that lowlands, seaports, the deltas of rivers and the neighborhood of marshes, are favorable places for the occurrence of the disease, while it seldom prevails extensively inland, and almost never at high altitudes.

If, in addition to this, we consider that it is a tropical disease, only extending as far north as our Gulf States and Charleston in the summer, usually about August, and dying out with the coming of frost (6), we have one of the reasons for likening it to yellow fever. Epidemics have possibly occurred in Philadelphia in 1780, and Ohio, in 1828, though we have not had access to the original accounts of these outbreaks, but it was only in the hottest part of the year in each instance.

Guiteras and Cartaya (7) say: "Dengue presents in its epidemiology a great resemblance to mosquito-borne disease, especially yellow fever. Epidemics appear in the hot season, even in the Tropics. It selects by preference the great seaports, the coast, and avoids the interior highlands. We meet places in which infection persists, epidemics which develop slowly in the beginning. If it then spreads rapidly it is because of the shortness of the incubation and the presence of many susceptible people. * * * Everything indicates that its transmission is not direct from sick to well. * * * It is common for a sick person to be taken to a place where dengue does not exist, and no harm result. * * * In Havana itself, and at the very height of the epidemic, there was a place in which the disease did not spread, simply because of freedom from insects. In Las Animas (Hospital) we have treated a good number of cases in the same rooms with other patients * * * without any contracting dengue. We have treated the greater part of our cases in the pavilion used for infectious diseases of known means of transmission, a pavilion protected against insects by metallic screening and frequent fumigation to kill mosquitoes, but where other disinfection was not practiced, and still dengue has not been transmitted to anyone."

They also speak of some experiments they made on mosquito transmission, but they do not publish the work, and state that they attach little value to it.

Cazamain (8), in reporting an epidemic on the French ship *Kersaint*, suggests the possibility of the mosquito acting as a "carrier."

The Australian committee (Robertson) report (9) says: "It is undoubtedly highly infectious, but clinical observation does not enable us to form any definite opinion as to the mode in which the infection is carried from person to person. In some respects the spread of the disease suggests some peculiarity in the method of propagation differing from that of the well-known diseases, influenza, scarlet fever, measles, etc. It appeared to spread particularly to contiguous houses, whole streets being attacked *seriatim.* One observation appears to have some bearing on the method of propagation. That is, the medical men have, in many instances, appeared immune to the disease, although exposed to it daily, indeed hourly, until cases appeared in their own households, when they fell victims, being usually among the last members of the households to be attacked."

Hirsch (10) says that its infectiousness is certain, its contagiousness doubtful, but he gives some significant quotations that suggest mosquito transmission. Thus he quotes Waring (North Amer. M. & S. Jour., 1830) as saying of the epidemic of 1826, "the breakbone fever has been suppressed by the frost," and of that of 1828, "it terminates under the effect of frost." Arnold (11) he quotes as follows: "This disease is undoubtedly affected by frost. The diminution of cases last fall was as marked as the diminution in our endemic climate [yellow] fever usually is."

Speaking of the Madras epidemic of 1872 Hirsch says that it ended in the middle of October with the onset of strong winds and colder weather. He also cites instances to show that freedom from epidemics is conferred by altitude.

Leichtenstern (12), although regarding the disease as contagious, makes the following suggestive observations concerning it:

1. It is a disease of tropical and subtropical zones, and in these zones it has a marked preference for the hot season, and almost always ends, as if suddenly cut off, on the recurrence of cold weather or the beginning of the cool season.

2. It is a disease of sea coasts and ports, and coast cities, and may go up large navigable streams, as the Mississippi. It rarely occurs inland or at high altitudes. When it went 4,000 feet high on Lebanon in 1889 the season was exceptionally hot.

3. He considers it a "highly contagious" disease, carried by ships, pilgrims and emigrants; nevertheless, contagion alone, in the strict sense of the term, can not satisfactorily explain its occurrence and spread.

4. He calls it contagious-miasmatic; that is, it is contagious from person to person, but only under certain conditions of time and place.

5. He likens its manner of spread (he wrote prior to the work of Reed, Carroll, *et al.*) to that of yellow fever.

Sandwith (13), writing of the disease in Egypt, says it always begins in August and September, with the rains, and ends in December, when colder weather begins. In speaking of its relation to surface water he says: "The conclusion would seem to be that dengue, with its unexplained affection for coasts, rainy seasons and large rivers, only appears in Egypt when the Nile is in its annual flood." He believed the disease to be contagious but cites instances that appear to point to some other method of diffusion.

Smart (14) likened the disease in some ways to yellow fever but said: "There is sufficient evidence of its infectious, if not of its contagious properties."

Fayrer (15) says: "The degree of communicability as well as the work of communication can, however, hardly be regarded as a settled question." He quotes Charles as expressing "his belief that when the eruption is out the danger of communicability is greatest and that on or about the tenth day, when the eruption has disappeared and after carbolic-acid baths, the danger of communication ceases." We think that this observation is probably correct, but explainable quite apart from any belief in direct contagion.

Fayrer quotes many Indian medical officers who express most diverse views concerning the epidemiology of the disease, yet nearly all seem to be readily accounted for under a theory of mosquito transmission.

Manson (16) does not express himself as believing in any special method of propagation, though he quotes Graham's work.

Van der Burg (17) says that epidemics are most common in the hot, moist season, and cease when the weather grows cooler, especially if very heavy rains occur, accompanied by cool winds. He thinks that dengue is very probably contagious, but that the contagious principle is different in character from that of smallpox, epidemic influenza and like diseases. He states that unknown exogenous conditions are necessary for its spread, wherefore many regard it as a "*contagious-miasmatic disease.*"

Scheube (18) says that the question of contagion is still undecided, but he considers the disease one requiring unusual conditions for its spread.

Agramonte (19) expresses the belief that the disease may exist in a latent state for weeks, under conditions not as yet well recognized.

Stitt (20) says that the disease is not infectious in the same sense as is influenza.

Harris Graham (21) in 1903 expressed his definite belief that the disease is transmitted by mosquitoes, and cited some interesting experiments in support of his belief. This belief seemed well founded, and the experiments in support of it almost conclusive, but the value of his work was impaired by the fact that in the same paper he described as the etiologic factor an intracorpuscular "organism with amœboid movement, and in many ways resembling the *Plasmodium malariæ.*"

It is probable that many workers, having satisfied themselves that such an organism did not exist in the blood of dengue, concluded that Graham had worked with some other disease, and so did not recognize the real importance of his work. We are unable to believe that the disease is characterized by the presence of any organism microscopically resembling that of malaria, but we think that our observations do lend support to Graham's conclusions as to mosquito transmission.

Carpenter and Sutton (22) failed to transmit the disease by means of mosquito bites, but we think that we can explain their failure more readily than we can the negative results which we obtained in some of our own experiments.

2. THE FORT WILLIAM M'KINLEY EPIDEMIC.

The epidemic from which we drew our cases is of great interest, as we think that its history fully supports our belief that the disease is mosquito-borne. Fort William McKinley is situated about 5 miles from the city of Manila, upon rolling land consisting of slight elevations interspersed with low, damp country. There are stationed at this post two regiments of infantry, two squadrons of cavalry, one battery of field artillery and one company of engineers.

The infantry barracks are situated near a small stream which drains the lowest part of the post in their vicinity. This stream is overgrown with a rank tropical vegetation and is an ideal breeding-place for mosquitoes. The epidemic began in the barracks of the Sixteenth Infantry, which is situated nearer this stream than any of the other barracks of the post. From the Sixteenth Infantry the infection spread to the Thirteenth, and from the Thirteenth to the contiguous barracks of the Eighth Cavalry. It is significant that the battery of Field Artillery and the company of United States Engineers almost entirely escaped the infection, and that their barracks are situated at least 2 miles from this stream, upon high, well-drained land.

The tables, and map which follow show the manner in which the disease spread from barrack to barrack and through the post. The stream mentioned is indicated upon the map in red.

Table illustrating the spread of dengue fever at Fort William McKinley from July 1, 1906, to November 1, 1906.

SIXTEENTH INFANTRY.							
Organization and date.	No. of cases.	Organization and date.	No. of cases.	Organization and date.	No. of cases.	Organization and date.	No. of cases.
Company A	20	Company C	25	Company D—Continued.		Company K[a]	------
July 1	1	July 2	2	October 28	1	Company L	24
July 2	1	July 12	2	Company E	1	July 13	1
July 3	1	July 16	1	July 22	1	July 15	1
July 8	1	July 18	1	Company F	3	July 17	1
July 9	1	July 20	1	July 17	1	July 18	1
July 10	1	July 21	1	August 5	1	July 22	1
July 12	1	July 22	1	August 9	1	July 24	1
July 13	2	July 23	1	Company G	7	July 25	1
July 15	2	July 24	1	July 20	1	July 27	1
July 17	1	July 25	3	July 23	1	July 30	1
July 18	1	July 26	2	August 6	1	August 1	1
July 22	2	July 28	2	August 7	1	August 5	1
July 25	1	August 1	1	August 8	1	August 6	2
July 27	1	August 2	2	August 9	2	August 9	1
July 30	1	August 5	1	Company H	8	August 11	4
August 1	1	August 11	1	July 18	1	August 14	1
August 7	1	August 16	1	July 24	1	August 20	1
Company B	26	August 24	1	August 2	2	September 9	1
July 7	1	Company D	24	August 4	1	September 10	1
July 9	1	July 5	1	August 5	1	September 14	1
July 10	2	July 12	1	August 7	2	September 20	1
July 11	2	July 14	2	Company I	12	Company M	8
July 12	2	July 16	1	July 26	1	July 25	1
July 13	4	July 17	1	July 30	1	July 30	1
July 15	1	July 18	4	August 4	1	August 3	1
July 16	1	July 19	3	August 9	1	August 7	2
July 17	1	July 20	1	August 14	1	August 24	1
July 18	1	July 23	1	August 15	1	August 28	1
July 19	1	July 26	1	August 18	1	August 29	1
July 20	1	July 27	1	August 19	1	Band	3
July 21	1	July 29	1	August 21	2	July 29	1
July 22	1	August 1	1	August 22	2	September 11	1
July 23	3	August 4	1			October 6	1
July 27	1	August 7	1			Total	161
August 1	1	August 8	1				
August 3	1	September 10	1				

[a] Not on duty at post.

Table illustrating the spread of dengue fever at Fort William McKinley from July 1, 1906, to November 1, 1906—Continued.

THIRTEENTH INFANTRY.

Organization and date.	No. of cases.
Company A	4
July 29	1
July 30	1
August 4	1
August 9	1
Company B	19
July 12	2
July 16	1
July 17	1
July 22	1
July 24	1
July 27	3
July 28	1
July 29	4
July 30	1
August 1	3
August 9	1
Company C	19
July 7	1
July 10	1
July 12	1
July 14	2
July 15	4
July 18	2
July 19	2
July 22	1
July 29	1
July 30	1
August 2	1
August 6	1
August 12	1
Company D	26
July 13	1
July 15	1
July 16	1
July 17	2
July 27	1
July 28	3
July 29	8
July 30	2
August 1	1
August 4	3
August 6	1
August 11	2
Company E	16
August 14	1
September 12	1
September 21	1
September 26	2
September 27	1
Company E—Continued.	
October 2	1
October 6	1
October 10	1
October 12	1
October 24	1
October 27	1
October 28	3
October 31	1
Company F	7
August 4	1
August 22	1
August 30	1
September 6	1
September 16	1
September 26	1
September 29	1
Company G	8
August 7	1
August 9	1
August 10	1
September 2	1
September 5	1
September 10	1
September 29	1
October 11	1
Company H	35
August 11	1
August 18	1
August 19	3
August 27	1
September 2	1
September 3	1
September 4	2
September 6	1
September 7	1
September 9	2
September 11	1
September 18	1
September 19	1
September 20	1
September 21	1
September 22	2
September 26	2
September 28	3
September 29	3
September 30	1
October 1	1
October 8	1
Company H—Continued.	
October 10	1
October 12	1
October 20	1
Company I	23
July 27	1
July 29	1
August 1	1
August 4	2
August 15	1
August 19	1
August 22	1
August 26	1
August 28	1
August 30	1
September 3	2
September 4	1
September 6	1
September 8	1
September 10	1
September 12	1
September 13	1
September 16	1
September 20	1
September 30	1
October 25	1
Company K	35
July 14	1
July 22	1
July 25	2
July 27	1
July 29	1
August 1	1
August 2	1
August 4	2
August 8	1
August 10	1
August 11	1
August 13	1
August 16	2
August 17	1
August 20	2
August 22	1
August 24	1
August 26	1
August 29	2
September 4	5
September 5	1
September 6	1
September 8	1
Company K—Continued.	
September 10	1
September 20	1
October 17	1
Company L	27
July 13	1
July 15	3
August 1	2
August 2	1
August 8	1
August 11	1
August 26	1
September 1	3
September 2	1
September 3	1
September 4	1
September 5	1
September 6	1
September 10	1
September 12	1
September 16	1
September 18	1
September 19	1
September 24	1
September 26	1
October 2	1
October 5	1
Company M	11
July 15	1
July 29	1
August 2	1
August 4	2
August 17	1
August 20	1
August 26	1
September 18	1
September 23	1
October 25	1
Band	8
July 18	1
August 8	1
August 11	1
August 13	1
September 18	1
October 1	1
October 15	1
October 24	1
Total	238

Table illustrating the spread of dengue fever at Fort William McKinley from July 1, 1906, to November 1, 1906—Continued.

EIGHTH CAVALRY.

Organization and date.	No. of cases.
Troop A	18
August 1	1
August 17	1
August 19	1
August 20	3
August 21	4
August 22	1
August 24	1
August 26	2
August 27	1
September 1	1
September 4	1
September 23	1
Troop B	12
August 1	1
August 2	1
August 18	2
August 24	1
September 6	1
September 12	1
September 16	1
Troop B—Continued.	
September 18	1
September 26	2
September 28	1
Troop D	16
July 29	1
July 30	2
August 2	1
August 6	2
August 10	1
August 18	1
August 19	2
August 22	1
August 23	1
October 1	1
October 2	1
October 22	1
October 23	1
Troop E	9
September 3	2
Troop E—Continued.	
September 5	1
September 6	1
September 11	1
September 23	1
October 7	1
October 27	1
October 29	1
Troop F	29
July 29	1
July 30	1
August 1	1
August 2	1
August 29	2
August 30	2
September 1	3
September 3	1
September 4	2
September 5	1
September 6	2
September 7	1
Troop F—Continued.	
September 16	1
September 17	1
September 18	2
September 19	1
September 21	1
September 23	2
September 30	1
October 1	1
October 20	1
Troop H	3
September 8	1
September 15	1
September 23	1
Band	3
August 2	1
August 14	1
October 15	1
Total	90

Table showing the strength of each company, the number of cases of dengue fever, and the percentage infected in the Sixteenth and Thirteenth Regiments of Infantry.

Name of organization.	Sixteenth U. S. Infantry.			Thirteenth U. S. Infantry.		
	Strength.	No. of cases.	Percentage infected.	Strength.	No. of cases.	Percentage infected.
Band	35	3	8	36	8	22
Company A	50	20	40	62	4	6
Company B	52	26	50	62	19	30
Company C	50	25	50	58	20	34
Company D	54	24	44	55	27	49
Company E	56	1	1	60	16	26
Company F	57	3	5	60	7	11
Company G	51	7	13	54	8	14
Company H	59	8	13	60	35	48
Company I	50	12	24	50	23	46
Company K	------	------	------	58	35	60
Company L	51	24	48	57	27	47
Company M	48	9	18	55	11	20

Table showing the strength of each company, the number of cases of dengue fever, and the percentage infected in the Eighth Cavalry.

Name of organization.	Eighth U. S. Cavalry.		
	Strength.	No. of cases.	Percentage infected.
Band	39	3	7
Troop A	56	18	32
Troop B	59	12	20
Troop D	56	16	28
Troop E	55	9	16
Troop F	55	29	52
Troop H	58	3	5

From the above tables it will be seen that the greatest ratio of infections in any one company was in Company H of the Thirteenth Infantry where 58 per cent of the men were infected, while in Company E of the Sixteenth Infantry only one man was infected, but this company left the post during the early days of the epidemic and thus can not be considered in this relation. It should also be remembered that Companies A, B, C, D of the Thirteenth and E, F, G, H of the Sixteenth were absent from the post after August 12, 1906.

In considering this table it will be noticed that the epidemic of dengue began in the Sixteenth Infantry upon July 1, reached the Thirteenth Infantry July 7, and the Eighth Cavalry not until July 29. It should be stated that the barracks of the Eighth Cavalry are at a considerable distance, at least 1 mile, from those of the Sixteenth Infantry, which, if we consider the disease to be mosquito-borne, will account for the long period of time elapsing between the infection of the two organizations.

On August 1, 1906, the First Battalion of the Thirteenth Infantry and the Second Battalion of the Sixteenth were ordered for field service in Leyte, P. I., and were still absent on this duty on November 1, 1906. At the time the First Battalion of the Thirteenth Infantry left Fort McKinley, 58 men had been sent to the post hospital suffering from dengue. No cases developed in this battalion on its voyage by boat to Leyte, nor have any cases developed in the battalion in Leyte. If dengue were a contagious disease this certainly would not have been the case, but removal from the focus of infection and from the disseminator of the infection—that is, the mosquito—resulted in a complete disappearance of dengue from this battalion.

At the time the Second Battalion of the Sixteenth Infantry left Fort McKinley, on August 12, dengue had just appeared, this battalion only having sent about 20 cases to the post hospital suffering from the disease; three days before leaving it sent 5 more, but no cases developed upon the voyage to Leyte, nor have any cases appeared in this battalion since arriving in Leyte. It seems to us that the almost instant disappearance of dengue from these battalions upon their removal from Fort McKinley and their voyage by sea to Leyte is almost positive proof of the relation of the mosquito to the transmission of the disease. It is obvious that no mosquitoes were present upon the boat, and that, as probably no cases were infected previously to embarking, the mosquitoes in Leyte did not become infected, and therefore dengue did not continue in these troops.

Another fact of importance in considering the transmission of this disease is the manner in which it spread from barrack to barrack. If the disease were purely contagious we would expect contiguous barracks to become infected in order. As a matter of fact, as shown by the map, in which the barracks are numbered in the order in which they became infected, contiguous barracks did not always become infected in the order one would suppose; thus, of two barracks standing side by side, one might be infected two or three weeks before the other, whereas another several hundred yards distant from the first might be the second one to become infected. A careful study of the map will show that this is true in a great many instances, and it can be explained, we think, by the erratic flight of an infected insect, such as the mosquito.

Lack of contagion is strongly suggested by the fact that although over 400 cases of dengue were treated in the general medical wards of the post hospital, and although no special means were employed to prevent contagion, no cases developed in the wards except in three attendants who were on night duty.

In our own camp, where the patients under our observation were treated, we have had 128 cases. Here no person developed the disease, except after inoculation and in consequence thereof, though attendants and patients mingled freely, and we made every effort, as will be seen in the detailed report of cases, to convey the disease by fomites, or air. We had dengue patients and well men sleep together, eat together and wear one another's clothing; both sick and well meanwhile abstained from bathing, and used a common close-stool kept in the tent with them. All the sick recovered and none of the well men developed the disease throughout the experiments, unless they were inoculated later.

We therefore feel justified in stating that *dengue is not contagious,* and think that the history and epidemiology, taken in connection with Graham's work and what we later detail, justify the correlative statement that *the disease is mosquito-borne.*

III. ETIOLOGY.

1. HISTORICAL.

The etiological factor concerned in the causation of dengue has been long and patiently sought for by almost every investigator who has studied the disease; thus, nearly every fluid, secretion and excretion of the body has been examined, and, as might be expected, not a few observers have announced, from time to time, the discovery of a causative organism. Many of these so-called "discoveries" were made during the dawn of bacteriological science, and a perusal of the methods adopted in isolating the organisms described, as well as the description of their morphology is sufficient to prove their absolute lack of scientific accuracy. Therefore, we have not deemed it necessary to review such contributions, but among the many who have investigated the etiology of this disease there are a few whose observations demand consideration. Among these may be mentioned McLaughlin, Graham, Carpenter and Sutton, Guiteras and Agramonte.

The early investigations regarding the etiology of dengue were directed toward a bacterial cause, but within the past few years the increasing importance of the protozoa in the etiology of disease has turned the current of investigation, and almost all of the work which has been done upon this subject by recent investigators has been founded upon the belief that the disease is caused by some protozoön, probably by one inhabiting the blood.

However, despite the fact that a great amount of labor has been expended in the search for a bacterial or protozoal organism, it is surprising how few records of this work there are and, upon analysis, how unsatisfactory the conclusions arrived at. Perhaps, in no other disease, as common and as thoroughly studied clinically as dengue, is there so limited a literature concerning its parasitic etiology and it must be admitted that this is one of the infections of man which, up to the present, has baffled all attempts at a solution of its etiology.

The earliest attempt, based upon bacteriological methods, to discover the organism causing dengue was that of McLaughlin (23). The descriptions of his experiments are detailed and the results obtained are of interest, but, viewed in the light of the approved bacteriological methods of to-day, are open to very severe criticism and have not been confirmed by late observers.

McLaughlin, whose observations were published in 1886, believed in the directly contagious character of the disease and that organisms of a bacterial nature, existed in the blood of the infected individual. He examined both fresh and stained specimens of blood, and cultures from blood made upon nutrient gelatine. He also made microscopical examinations of the vomitus and urine and endeavored to secure cultures of the organism by partially filling sterilized glass bulbs with blood from a vein and allowing the blood so obtained to incubate for weeks and even months. By this method he examined but one case, but by the methods previously given he studied forty. In the blood of every case he found spherical micrococci measuring one-twentieth to one-thirtieth the diameter of a red blood corpuscle, and red or purplish in color; in preparations made from cultures grown upon nutrient gelatine, the cocci, when in masses, appeared black or brown, but when seen singly the red color was always distinct and characteristic. In the bulbs mentioned, which contained only blood (no bouillon), the same organisms were found in pure culture after an incubation period of from six weeks to three months.

While the researches of McLaughlin appear to have been partially accepted, or at least considered seriously by some writers, we regard them as only of purely historical interest, for reasons which are obvious.

Klein investigated very carefully the report of the presence of a short bacillus in the blood of dengue cases and concluded that there was not sufficient evidence to prove the constant association of this or any other organism with the disease. Wright was unable to demonstrate any organism as concerned in the etiology of dengue, and similar results followed the investigations of Crookshank and MacFadyen.

The observations of Graham (21) of Beirut, published in 1903, awakened increased interest in the etiology of this puzzling disease, and a considerable amount of work has since been done with the object of confirming or disproving his results. Graham's investigations were carried out in Beirut, Syria, and resulted in the announcement by him that dengue is due to a protozoön inhabiting the red blood corpuscles ~~and~~ closely resembling the plasmodia of malaria, except for the absence of pigment. He examined the blood of over one hundred cases, but in his communication regarding the subject he does not state in how many of these he found the parasite, but admits that he was unable to demonstrate it in stained smears of the blood. As described by Graham, the parasite first appears as a small, hyaline rod or dot within the red blood corpuscle, constantly changing its position, the motility in some instances being very marked; later, the parasite increases in size, appears to present typical amœboid motion, and finally almost fills the red corpuscle, or rupturing it becomes free in the plasma, where it very soon degenerates. No sporulating forms are described, nor any other method of reproduction in man. The organism is never observed to contain any pigment. He states that he has found the same bodies in the blood of patients suffering from second and third attacks of the disease.

From the results which this investigator obtained in transmission experiments to be mentioned later, he believed that the organism described underwent a developmental stage within the mosquito, and he therefore endeavored to trace such a cycle, using mosquitoes of the genus *Culex fatigans* Wied. for the purpose. By dissecting insects which had bitten dengue patients and examining the blood contained in the stomach, he claims to have demonstrated his piroplasma-like bodies in the mosquito up to the fifth day after the biting, and states that they undergo developmental changes similar to those occurring in the blood corpuscles in man. He did not succeed in finding any *zygotes* or any evidence of sexual forms within the mosquito. He also claims to have observed the spores of this organism "in among the cells of the salivary glands" after forty-eight hours in mosquitoes which have bitten a dengue patient upon the fourth day of the disease; he further claims that the spores could be demonstrated in the salivary glands of mosquitoes which had been kept a month.

Graham produced a very severe case of fever resembling dengue by inoculating a man subcutaneously with peptonized normal salt solution containing the salivary glands of a mosquito which had bitten a dengue patient twenty-four hours before. He was deterred from further experimentation along this line by reason of the very severe symptoms produced in this case.

Because of the positive character of Graham's statements his work attracted widespread attention and encouraged many investigators to study with renewed energy the etiology of dengue, but although many experienced microscopists have endeavored to confirm his results none have done so as regards the presence of a parasite within the blood corpuscles. With the exception of Eberle, whose description of a *plasmœba* obviously applied to vacuoles, artefacts, etc., which are so common in the blood of fever patients, no investigator claims to have been able to demonstrate in dengue blood in nature, any constant parasite, bacterial or protozoäl.

Carpenter and Sutton (22) studied 200 cases of dengue upon the Isthmus of

Panama, and examined both fresh and stained specimens of blood, using Wright's, Ehrlich's, the Romanowsky and Nocht's methods, as well as bacterial stains. They were unable to demonstrate McLaughlin's micrococcus or any other microörganisms in the blood. They found the nonpigmented bodies of Graham in unstained specimens, but not in stained ones. They also found the same bodies in the blood in other diseases; and regarded them as being due to necrobiotic changes in the red cells. They also examined mosquitoes which had bitten dengue patients, but found no parasites present in the insects.

Guiteras (7), as the result of a very careful investigation in Habana in 1905, believes that Graham is mistaken regarding his organism and concludes, after examining a large series of blood specimens taken during all days of the disease, at various hours and stained by various methods, that the blood contains no structure resembling a parasite.

The investigations by the staff of the Government Laboratories (24) at Manila, P. I., in 1900 into the etiology of dengue, resulted negatively as to the presence of a parasite. Their conclusions were as follows: (1) In dengue fever there is no leucocytosis; (2) the differential count of the white corpuscles in this disease show normal proportions of the several varieties; (3) the hæmatozoön described by Graham has not been found present in the circulating blood of our cases; (4) the micrococcus described by McLaughlin has not been encountered.

Agramonte (19), studying the disease in Habana in 1906, was unable to demonstrate any parasite in the blood, and the recent researches of Kieweit de Jonge and de Haan (25), in Java, which were most thorough, were also without result. Stitt (20), working upon the subject in Cavite, P. I., was unable to demonstrate any organism in the blood.

Method of transmission.—Until the publication of Graham's work practically nothing had been done in the way of experimental research directed toward the discovery of the method of transmission of dengue fever. We have already touched upon the theories regarding this question in considering the epidemiology of the disease, but so far as we have been able to determine, after consulting all the literature available, to Graham belongs the credit of first attacking this problem in a practical and scientific manner. His work aroused much interest, and however he may have erred in his interpretation of the bodies described by him as the cause of the disease, we believe that his experiments regarding the method of transmission are most valuable and his conclusion that dengue is transmitted by the mosquito is well founded and has been experimentally confirmed.

Graham's experiments regarding mosquito transmission were briefly as follows: Four men in good health were selected and slept night after night beneath mosquito bars containing mosquitoes that had bitten dengue patients. In one case the disease developed four days from the date of the first exposure, in one in five days, and in one in six days. In one case the result was negative. During the time the experiment lasted the men remained in their homes, where there had been no other cases of the disease, and where none developed later. In order to obviate the possibility that these men might have contracted the disease in some other way, there being a very severe epidemic in the city at that time, Graham took mosquitoes that had bitten dengue patients to a village situated in the mountains, where no cases of the disease had occurred. At this village he liberated the mosquitoes under the nets of two young men, living in different localities, and orders were given that the men were not to leave the nets until permitted. One of these men developed a very severe attack of dengue in four days after exposure, the other in five days. The mosquitoes were destroyed and the men continued to sleep under the mosquito bars for some time after recovery. No other cases of the disease occurred in this village. The mosquitoes used in these experiments are stated by Graham to be *Culex fatigans* Wied., and the insects were used within a short time after biting infected patients. Graham further states that in Beirut no *Anopheles* are to be found, but that during the dengue epidemic the city was infested with great numbers of *Culex fatigans* Wied. It will be seen that of six healthy men bitten by infected mosquitoes, five developed dengue, two in four days, two in five days, and one in six days.

Since Graham's experiments and results were published, a few observers have endeavored to confirm them, but without success. Carpenter and Sutton (22) experimented with various mosquitoes, viz: *Culex stimulans* Walk., *C. tarsalis* Coq., *Stegomyia fasciata* Fbr., etc., but not with *Culex fatigans* Wied. The mosquitoes used were young, well-grown insects, reared by them and kept for from five to fourteen days after biting, when they were dissected and very carefully examined. No protozoa were demonstrated, nor any other organism which they regarded as of etiologic importance. The authors regard their mosquito inoculation experiments as untrustworthy, and state that no definite statements can be made concerning them. They give but four experimental cases of mosquito inoculation, two of which were negative, one positive after six days and one after two weeks from the date of mosquito bites; the latter case is obviously of no value, as the incubation period is too long. The authors state that "the volunteer subjects were not only exposed to the bites of other moquitoes at all times but they were also brought into almost daily contact with dengue cases.

Agramonte (19), in Habana, attempted to transmit the disease by mosquitoes, trying various species at various intervals after the insects had fed upon dengue patients, but was unsuccessful in producing the disease in this way. He believes, however, that dengue is transmitted by some species of mosquito, and that the reason for failure in experimental infection lies in some undiscovered fault in technique. The epidemic he studied was accompanied by a plague of *Culex fatigans* Wied.

In Habana, Guiteras and Finlay (7) endeavored to transmit the disease with *Culex pipiens* Linn., but with negative results. Guiteras states, regarding these experiments, that their small number and lack of variety deprive the negative results of a claim to conclusive character, and that their faith remains unshaken that the mosquito is the transmitter of dengue.

It is rather surprising, in view of the scientific interest and importance of this question, that so little has been done to prove or disprove the results claimed by Graham from his mosquito experiments; it is not, however, surprising, that those who have attempted to solve the problem have met with so little success, for only those who have done so can realize the difficulties and disappointments which await the investigator in this particular field of research. In our work upon this phase of the subject, to be detailed later, the experimental results obtained, while to our own satisfaction proving that *Culex fatigans* Wied. transmits dengue fever, have been, at times, most disappointing and discouraging; unknown natural conditions appear to be necessary for the transmission of this disease by the mosquito, and because of our ignorance of the method of securing these we believe that experimental transmission by the mosquito is rendered most difficult and that negative results may be expected much more frequently than positive ones.

2. EXAMINATION OF THE BLOOD.

In attempting to solve the etiology of dengue and its method of transmission, our attention was first directed to the microscopical examination of the blood of patients suffering from the disease. We have already noted the failure of numerous observers to confirm the presence of McLaughlin's or Graham's organisms, and also their negative results as to other blood parasites. Despite these we considered that our work would be incomplete without careful examination of both fresh and stained preparations of the blood, and accordingly we have studied thoroughly, in this respect, a large number of our cases; the blood was examined during every period of the disease, but especially during the first two days and during the terminal rise in the temperature; various staining methods were used, including Wright's stain and the methods used in demonstrating *Treponema pallidum.* The latter methods were used very carefully and in numerous cases, as at the time we began our work we were greatly inclined to believe that the organism concerned in the etiology of dengue might belong to the spirochætæ. We have not been able to confirm the results of McLaughlin or Graham, nor have we been able to demonstrate any organism in the blood of dengue patients which we can consider as an etiological factor.

There is but little in the literature concerning the changes in the blood in this disease, and even Graham's claim that the disease is due to a hæmatozoön which destroys the red corpuscles does not seem to have stimulated research in this direction. We shall, therefore, in this portion of our report, detail the results of our study of the blood which demonstrate that, whatever the cause of dengue may be, it is not an organism that influences to any marked extent the essential characteristics of the blood, with the exception of the relative proportions of the various forms of leucocytes.

(*a*) *Hæmoglobin.*—In uncomplicated cases the hæmoglobin and color index are normal.

Erythrocytes.—Number: Dengue is not a disease in which anæmia is present. We have made numerous blood counts in severe cases, and have never observed a count lower than 4,500,000 red cells per cubic millimeter, even when the count was made at the termination of the disease. This fact alone appears to us conclusively to disprove the existence of Graham's hæmatozoön, which by destroying the red blood corpuscles during its development within them would certainly reduce their number. We have never seen a case of uncomplicated dengue in which the clinical symptoms suggested anæmia. Our observations are borne out by those of Carpenter and Sutton (22), who found that the red blood count in dengue was generally over 5,000,000 per cubic millimeter.

Morphology: In size the red blood corpuscles are unchanged. Poikilocytosis is not commonly observed, but in some cases, during the height of the fever, a moderate degree of poikilocytosis may be present. Crenation does not occur more rapidly nor is it more marked in dengue than in other acute, febrile conditions. Vacuolation is common both in fresh and stained specimens of blood, and in many instances the shape and appearance of the vacuoles is very suggestive of a parasitic invasion of the red cell; artefacts, due to degeneration of the protoplasm and clear areas due to retraction of the hæmoglobin, are common, especially in poorly prepared smears and are well calculated to lead to error because of their resemblance to bacterial or protozoal organisms. We have not observed that the appearance of the vacuoles occurring in the red corpuscles in dengue differs from that seen in many other febrile conditions, but it is certainly true that they frequently present an appearance very suggestive of amœboid motion without change of position; the progressive motion referred to by some writers which we have observed in the case of rod-shaped artefacts, is probably due to protoplasmic currents within the degenerating red cell.

It is not uncommon to observe in the blood of dengue, as well as in that of other febrile conditions, cocci or bacilli, either free in the blood plasma or attached to the red blood corpuscles; in the vast majority of instances these bacteria are due to external contamination and have no relation to the disease in which they are observed; when they are attached to the red blood cell and still possess some motility their resemblance to a parasite is often striking, but it is possible by gentle pressure to dislodge them and thus demonstrate their real nature.

We have not observed the presence of normoblasts or megaloblasts in the blood in dengue, and their absence, especially of normoblasts, indicates that anæmia even of a mild type, is not present.

The staining reactions of the red corpuscles in dengue do not differ from those present in health. Polychromatophilia or basophilia we have not observed, but in poorly prepared specimens the staining may be irregular, suggesting granular degeneration.

From our observations we conclude that the morphology of the red cell in dengue shows no diagnostic changes.

(*c*) *The leucocytes.*—Number: One of the most important blood changes in this disease is the presence in almost every case of a marked leucopenia. From our observations we are convinced that the leucopenia of dengue is almost constant throughout the attack, and that it is of considerable diagnostic importance. We have made leucocyte counts in a large number of cases and have invariably found a marked reduction in the total number with, as will be seen later, a quite characteristic change in the relative proportion of the various forms. The lowest leucocyte count was 1,200 per cubic millimeter, the highest 4,860, the average, 3,800. Carpenter and Sutton (22) found a constant leucopenia, the lowest count being 1,866, the highest 5,866, the average about 3,500, per cubic millimeter. Stitt (20) states that a leucopenia is always present, and his counts varied from 1,450 to 5,280 per cubic millimeter. We have found that the leucopenia is progressive, being most marked upon the fifth or sixth day of the disease.

Morphology: We have observed no morphological changes in the leucocytes, nor any evidence of the presence of a leucocytozoön.

Differential blood count: From the studies of Carpenter and Sutton, and later of Stitt, the differential leucocyte count in dengue has assumed considerable diagnostic importance, and taken together with the leucopenia appears to us to be entitled to very careful consideration in the differential diagnosis of dengue, yellow fever, malaria, and the eruptive fevers.

Carpenter and Sutton (22), from their examinations, conclude that in dengue there is always a leucopenia, and generally an increase in the small lymphocytes and in the eosinophiles, the latter occurring late in the disease.

Stitt (20) made differential leucocyte counts at varying periods of the disease. He found that a marked variation occurred in the different forms of the leucocytes at different periods, there being at first a large increase in the small lymphocytes, succeeded by a greater increase in the large lymphocytes, and finally, during the terminal eruption, a most marked increase in the large mononuclears.

Because of lack of time we have made comparatively few differential leucocyte counts, but our results have been supplemented by those of Lieutenant Vedder, Medical Department, United States Army, stationed at Fort William McKinley, who kindly volunteered to assist us in this direction, and whose tables and remarks are given below. From our own observations we are loath to lay much stress upon the variation in the relative proportion of the large and small lymphocytes, as does Stitt, for in many instances we have not found a constant relationship between the variety of lymphocyte which is increased and the period of the disease, but we have found a constant leucopenia, a decrease in the polymorphonuclears and an increase in small lymphocytes. In one of our experimental cases (Case 9) in whom we produced a severe attack of dengue by the intravenous inoculation of the filtered blood from another experimental case, the leucocyte counts made upon the first, third and sixth days of the disease, well illustrate the changes described by Stitt, as will be seen by the following record:

First day of disease:	
Polymorphonuclears	50
Small lymphocytes	41
Large lymphocytes	7.5
Eosinophiles	1.5
Third day of disease:	
Polymorphonuclears	52
Small lymphocytes	36
Large lymphocytes	8
Eosinophiles	4
Sixth day of disease:	
Polymorphonuclears	48
Small lymphocytes	14
Large lymphocytes	32
Eosinophiles	6

It will be observed that the eosinophiles showed a marked increase as the disease progressed, and this has been noticed in several of our cases. While the above differential count is typical of the results obtained by some observers, we have found, even in a limited number of examinations, that it is not of sufficiently frequent occurrence to be depended upon alone in reaching a diagnosis. In fact, in most of our counts we found that the small lymphocytes outnumbered the large in every stage of the disease.

Vedder, as a result of his observations upon the white blood count in dengue, has submitted to us the following data. His blood counts were made on patients suffering from the disease occurring in the same epidemic which we have described above. Regarding his results he says:

> The polymorphonuclear leucocytes are greatly decreased and the small lymphocytes are greatly increased, while the large lymphocytes are moderately increased during the latter days of the illness. These changes are, to a greater or less degree, characteristic of the disease throughout its duration. The decrease in polymorphonuclears and the increase in small lymphocytes takes place almost at once, being very noticeable on the second day of the illness.

Vedder regards the differential leucocyte count as of great value in the diagnosis between this disease and yellow fever.

The following table gives in percentages, the results obtained by Vedder in five cases in which a differential count was made on each day of the disease:

Day.	Leucocytes.	Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Mononoclears.	Eosinophiles.
CASE 1.						
	Number.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	5,300	79.50	16.00	2.50	0.50	1.50
2	4,100	50.00	43.00	5.50	1.50	0
3	5,900	45.00	48.25	5.75	1.00	0
4	4,400	46.00	40.25	10.75	3.00	0
5	4,700	58.25	34.00	6.30	1.25	0
6	5,250	46.33	43.33	8.66	1.00	.66
7	4,700	29.50	56.00	11.75	2.50	0
8	4,800	27.50	60.00	11.25	.50	.75
CASE 2.						
1	4,200	60.00	17.75	7.50	2.00	12.75
2	3,800	40.50	45.00	10.00	3.00	1.50
3	6,500	25.00	54.33	8.00	2.00	10.66
4	2,750	34.00	44.00	6.00	1.00	15.00
5	2,600	45.00	45.66	7.66	1.00	1.66
6	4,550	23.33	48.33	8.33	.66	19.33
7	----------	38.75	50.00	8.50	1.75	.50
8	5,550	30.00	47.33	6.33	.66	15.33
9	----------	30.00	46.66	11.66	1.33	10.00

CASE 3.

Day.	Leucocytes.	Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Mononoclears.	Eosinophiles.
	Number.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent*
1		67.50	25.25	6.24	1.00	0
2		50.66	43.33	4.66	1.00	.33
3		29.00	59.25	8.25	2.50	.25
4		27.00	52.00	16.00	2.50	1.50
5		30.00	55.00	10.00	2.00	3.00
6		--------	--------	--------	--------	--------
7		37.00	36.50	14.00	9.50	3.00
CASE 4.						
1		62.00	29.50	7.00	1.00	0.50
2		48.00	45.66	4.66	1.00	.33
3		54.50	37.00	5.00	2.50	1.00
4		56.50	33.50	3.50	1.00	5.50
5		38.00	50.00	8.00	1.00	3.00
6		34.50	47.00	11.50	2.00	5.00
7		41.00	36.75	11.25	3.50	7.25
8		28.66	43.33	12.66	3.33	2.33
CASE 5.						
1		75.33	16.66	6.00	0.33	1.33
2		45.66	46.00	5.33	3.00	0
3		48.75	45.25	4.50	1.00	.50
4		37.00	51.00	8.00	2.00	1.00
5		29.00	58.50	8.50	3.50	0
6		27.00	47.50	18.75	3.75	2.25
7		35.25	46.00	13.75	1.50	3.25

The following table gives the average of ten differential counts made on each day of the disease for eight days:

Table showing average of ten counts made on each day of disease for eight days.

FIRST DAY.

Count.	Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Mononoclears.	Eosinophiles.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	79.50	16.00	2.50	0.50	1.50
2	85.25	10.75	2.75	1.00	.25
3	60.00	17.75	7.50	2.00	12.75
4	78.00	17.33	3.00	1.33	.33
5	58.00	36.33	4.00	1.66	0
6	55.66	37.66	6.00	.66	0
7	68.75	22.00	6.75	2.25	.25
8	66.25	26.25	5.25	1.00	.25
9	72.75	23.00	3.00	1.25	0
10	67.50	25.25	6.25	1.00	0
Average	69.16	23.23	4.70	1.26	1.53

Table showing average of ten counts made on each day of disease for eight days—Cont'd.

Count.	Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Mononoclears.	Eosinophiles.
SECOND DAY.					
	Per cent.	*Per cent.*	*Per cent.*	*Per cent*	*Per cent.*
1	42.80	42.60	6.80	1.00	6.80
2	47.50	46.50	5.50	.50	0
3	27.50	65.75	5.00	.75	.25
4	50.00	43.00	5.50	1.50	0
5	49.00	44.00	6.00	.50	.50
6	40.50	45.00	10.00	3.00	1.50
7	48.00	39.00	9.00	3.00	1.00
8	32.66	59.00	6.00	2.33	0
9	50.66	43.33	4.66	1.00	.33
10	48.00	45.66	4.66	1.00	.33
Average	43.66	47.38	6.31	1.45	1.07
THIRD DAY.					
1	58.00	34.50	6.50	1.00	0
2	48.75	45.25	4.50	1.00	.50
3	54.50	37.00	5.00	2.50	1.00
4	29.00	59.25	8.25	2.50	.25
5	35.33	47.33	11.66	3.00	2.66
6	33.50	46.00	13.50	6.00	.50
7	51.00	33.66	12.66	2.00	.66
8	37.00	50.66	10.33	2.00	0
9	29.00	58.33	10.66	2.00	1.00
10	25.00	54.33	8.00	2.00	10.66
Average	40.10	46.63	9.10	2.40	1.72
FOURTH DAY.					
1	38.75	54.75	5.50	0.25	0.50
2	46.66	34.33	8.66	5.33	4.33
3	59.75	33.00	2.50	.25	4.25
4	34.66	57.33	5.66	.66	1.00
5	46.00	40.25	10.75	3.00	0
6	34.00	44.00	6.00	1.00	15.00
7	36.66	55.00	6.66	1.00	.66
8	27.00	52.00	16.00	2.50	1.50
9	32.25	54.75	10.00	3.00	.25
10	37.00	51.00	8.00	2.00	1.00
Average	39.27	47.64	7.97	1.89	2.84
FIFTH DAY.					
1	25.66	47.66	14.33	0.66	11.00
2	34.00	42.33	18.66	2.66	2.00
3	38.00	50.00	8.66	1.66	.33
4	59.75	33.00	2.50	.25	4.25
5	58.25	34.00	6.50	1.25	0
6	45.00	45.66	7.66	1.00	1.66
7	30.00	55.00	10.00	2.00	3.00
8	38.00	50.00	8.00	1.00	3.00
9	29.00	58.50	8.50	3.50	0
10	38.00	46.60	10.80	3.00	1.40
Average	39.56	46.27	9.56	1.69	2.66

Table showing average of ten counts made on each day of disease for eight days—Cont'd.

Count.	Polymorphonuclears.	Small lymphocytes.	Large lymphocytes.	Mononoclears.	Eosinophiles.
SIXTH DAY.					
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	17.60	70.60	10.00	1.80	0.80
2	33.00	49.00	12.75	3.75	1.00
3	45.75	41.75	8.50	1.25	2.50
4	55.00	35.00	7.40	.60	1.60
5	46.33	43.33	8.66	1.00	.66
6	23.33	48.33	8.33	.66	19.33
7	29.33	57.33	11.33	1.33	.66
8	34.50	47.00	11.50	2.00	5.00
9	34.00	56.33	7.66	1.00	.66
10	52.33	34.33	9.66	1.00	2.66
Average	37.11	48.30	9.57	1.43	3.48
SEVENTH DAY.					
1	29.50	56.00	11.75	2.50	0
2	38.75	50.00	8.50	1.75	.50
3	37.00	36.50	14.00	9.50	3.00
4	41.00	36.75	11.25	3.50	7.25
5	35.25	46.00	13.75	1.50	3.25
6	41.50	52.00	4.50	.75	1.25
7	31.75	60.75	6.75	.75	0
8	25.00	57.75	12.50	3.50	1.00
9	17.50	64.75	12.00	1.50	4.00
10	51.66	40.66	5.66	1.00	1.00
Average	34.89	50.11	10.01	2.62	2.12
EIGHTH DAY.					
1	27.50	60.00	11.25	0.50	0.75
2	44.00	35.66	14.33	2.66	3.00
3	30.00	47.33	6.33	.66	15.33
4	38.33	47.33	6.66	2.66	4.66
5	28.66	43.33	21.66	3.33	2.33
6	41.75	47.50	8.50	.50	1.50
7	42.33	37.33	13.33	3.66	2.00
8	48.66	35.66	11.66	.66	3.33
9	51.33	36.00	5.33	0	6.66
10	37.50	53.50	5.50	.75	2.50
Average	39.00	44.36	10.45	1.53	4.20

Summary.—From our examinations of the blood in dengue we consider that the following conclusions are justified:

1. There does not occur in the blood of dengue any visible organism, either bacterial or protozoal in nature, which can be considered as the cause of the disease. We have not observed any protozoön in the blood.

2. Dengue is not accompanied by anæmia, the red blood count being normal in uncomplicated cases. There are no characteristic morphological changes in the red corpuscles, leucocytes, blood plates or blood plasma.

3. Dengue is characterized by a leucopenia and in the vast majority of instances by a decrease in the polymorphonuclear leucocytes and a marked increase in the small lymphocytes; the increase in the small lymphocytes is constant throughout the disease.

(*d*) *Blood plates.*—We have observed no changes in the number or appearance of the blood plates in dengue.

(*e*) *The blood plasma.*—In neither fresh nor stained specimens of blood have we been able to demonstrate any organism of etiological significance in the blood plasma in dengue. In a few instances bacteria were noticed but from the ease with which blood cultures collected with the most careful attention and asepsis become contaminated in this climate, we believe that these bacteria were of external origin. The most common bacterium observed was a long, stout bacillus, actively motile, and commonly seen here in blood specimens from various sources. No organism resembling a protozoön was observed in the blood plasma. Yeast cells were frequent contaminations in stained specimens.

3. BLOOD CULTURES.

With the exception of McLaughlin's researches, which have been mentioned and which, so far as cultural methods are concerned, were almost valueless, we have not been able to find in the literature any detailed descriptions of experiments having for their object the cultivation of bacteria or protozoa from the blood of dengue patients. We therefore approached this portion of our task with high hopes that by applying modern cultural methods we might be able to isolate and cultivate the organism causing the disease. In view of the success attained by Novy and others in the cultivation of trypanosomes, and by Rodgers in cultivating the Leishman-Donovan body (*Herpetomonas donovani*), we were especially hopeful that by applying similar methods in this disease, we might be able to secure growths of any protozoön which might be present. As we have stated, we were impressed with the idea that dengue might be caused by an organism belonging to the same group as those producing relapsing fever, or to some closely allied group, and we therefore endeavored, both by employing special staining methods and culture media, to demonstrate such an organism.

(*a*) *Methods.*—In our experiments we have used citrated blood and acid and alkaline bouillon as culture media. In making cultures with citrated blood, the sterilized syringe was first filled with citrated solution, which was then ejected, a very little being allowed to remain in the needle; the syringe was then filled with blood by plunging the needle into a prominent vein of the forearm and withdrawing the blood very slowly until the barrel of the syringe was full; the blood was then ejected into small sterilized glass tubes and kept at room temperature in the incubator or in the lower compartment of an ice box, the latter in order to give any organism undergoing a part of its life cycle in a cold-blooded animal, surroundings congenial to its development.

In making blood cultures in bouillon, 10 cubic centimeters of blood obtained from the median basilic vein were added to 250 cubic centimeters of bouillon contained in 500 cubic centimeter flasks, and incubated at temperatures of from 26° to 36.05° C.

In preparing our cultures the utmost care was taken to avoid infection, everything being rendered sterile that could be, but despite all of our precautions the majority of our cultures sooner or later became contaminated with various forms of bacteria or yeasts.

(*b*) *Citrated blood cultures.*—In eight cases we have endeavored to secure cultures of the organism causing dengue by citrating blood obtained from dengue patients at various periods of the disease. In none of these have we been able to demonstrate any organism which we can consider to be of any etiologic significance; in none of the tubes of citrated blood did we encounter any organism resembling, in the least, a protozoön, and all of the bacteria observed were evidently contaminations. A small diplococcus occurred in two cases, but in the light of our later work on filtered blood this obviously is of no significance.

(*c*) *Bouillon blood cultures.*—In twelve cases we used bouillon blood cultures, allowing them to incubate for as long as eight weeks. The majority of the flasks became infected, but in four cases the blood cultures did not show any growth at the end of eight weeks, when they were destroyed. A staphylococcus grew in one in forty-eight hours, a diplococcus in three in seventy-two hours, accompanied by a large spore-bearing bacillus in two of the cases; a short, thick, motile bacillus, together with a staphylococcus in one, in four days and various spore-bearing baccilli in the remainder. These organisms we believed to be contaminations and therefore we did not perform any experimental work with them. Our conclusions regarding them were confirmed by the result of our experiments with filtered blood

As the result of our culture experiments we were forced to conclude that no organism was found constantly enough in the cultures to warrant us in regarding it as having an etiological relationship to the disease, especially as a number of them remained sterile, although kept for as long as eight weeks.

INOCULATION EXPERIMENTS.

Having thus failed to demonstrate any organism in either fresh or stained specimens of blood or in our blood cultures, we directed our attention to the possibility of producing the disease by the inoculation of blood from the dengue patient into the healthy man; fortunately for the success of our work we were dealing with a disease which, in the young and robust, is not dangerous to life, and for this reason we felt justified in making such experiments. We hoped in this way to determine the presence or absence of the infective agent in the blood, for should such experiments prove successful they would demonstrate that the cause of the disease is in the blood, and that therefore, insect transmission, is possible, whereas negative results would prove that the blood does not contain the organism unless it be one that first has to undergo a developmental cycle outside of the body, as in an insect, before it can produce the disease in man.

In order to secure subjects for experiment a call for volunteers was issued to members of the Hospital Corps serving at the United States Army Division Hospital, and four men volunteered, in all of whom we succeeded in producing dengue by the intravenous inoculation of blood from cases of the disease. We desire to express our admiration of the courage and devotion to duty of these men, who, with no prospect of pecuniary reward, cheerfully placed themselves in our hands for experimentation.

As more men were needed and as no more Hospital Corps men were available, we consulted Major General Leonard Wood, United States Army, commanding the Philippines Division, who authorized us to offer a reward for volunteering, as a result of which we secured many more volunteers than we needed and were forced to refuse a large number, as we were limited to sixteen, including the men we had already used. Unfortunately, of the fourteen men we have experimented upon, seven came from Fort William McKinley having passed unharmed through the dengue epidemic, and of these men we found two absolutely immune, three relatively immune, and one doubtful. Of the same number of Hospital Corps men who had not been exposed to dengue, we found only one immune.

Of the fourteen soldiers who volunteered for this work, seven belonged to the United States Army Hospital Corps, three to the Eighth United States Calvary, two to the Sixteenth United States Infantry, one to the Thirteenth United States Infantry, and one to Company B., Engineer Corps.

4. INTRAVENOUS INOCULATION WITH UNFILTERED DENGUE BLOOD.

Eleven of our fourteen volunteers were given intravenous inoculations of unfiltered blood from dengue patients, and of these, seven developed the disease, while in one case the result was doubtful. In three of the cases there existed, apparently, an absolute immunity to the disease. These cases will now be considered in detail.

Experiment No. 1.

Case 1, Chart 1.—E. U., private, Hospital Corps, United States Army. Had not been exposed to dengue. At 3.30 p. m., July 24, 1906, he was given an intravenous injection of 20 minims of unfiltered blood from Case 20 (see Chart A). The patient from whom the blood was taken for the inoculation had a mild attack of dengue and was probably nearly over the disease at this time. We believe that this accounts for the mild character of the experimental disease in this case, for while the symptoms which were present were typical, it will be seen on referring to the temperature chart that the fever was slight as compared with our other experimental cases, which were all inoculated with blood from more severe cases, taken at an earlier period of the disease. The following is a résumé of the clinical record in this case:

July 24: At 3.30 p. m. inoculated intravenously with 20 minims of blood from Case 20. Subject in good health and temperature normal.

July 25: Feels perfectly well.

July 26: Feels well. No symptoms.

July 27: Ditto.

July 28: Has some fever and headache. Bowels constipated.

July 29: Patient feels uncomfortable, complaining of vague muscular pain and burning and smarting of eyes; eats and sleeps fairly well. Bowels regular.

July 30: Last night felt very uncomfortable, having severe headache and pain in eyes; also pain in the lumbar muscles, ankles, elbows and wrists. At present (11 a. m.) complains of dull headache and slight lumbar pain; the eyes are painful, the pain being aggravated by movement. Tongue moist and clean. Bowels constipated.

July 31: Feels much better, the pains in the head and muscles having disappeared. A slight eruption is present upon the chest and back.

August 2: Feels well. Eruption has faded.

August 6: Returned to duty.

Remarks.—Upon reference to the temperature chart (Chart 1) it will be observed that the temperature began to ascend about 9 a. m. on July 28, but the patient complained of no symptoms until nearly twenty-four hours later. The incubation period in this case, therefore, is about three days and eighteen hours, reckoning it from the 9 a. m. temperature on July 28.

Experiment No. 2.

Case 2, Chart 2.—W. R. H., private, first class, Hospital Corps. Not previously exposed to dengue so far as known. In good health. At 11 a. m., July 31, 1906, was given an intravenous inoculation of 20 minims of blood from Case 30 (Chart B), who was suffering from a typical attack of dengue of four days' duration at the time the blood was obtained. No symptoms appeared in Case 2 until early in the morning of August 3, as is shown by the following résumé of the clinical record:

July 31: Inoculated with dengue blood as stated.

August 1 and 2: Feels well.

August 3: At 2 a. m. patient awakened by pain in chest and some difficulty in breathing. This soon passed away and patient slept until morning. Felt well upon awaking this morning but soon developed sharp pain in the head, muscles of the back and legs. Complains also of a slight cough and pain in the eyes. Had slight chill at 5.30 p. m. to-day. No appetite.

August 4: Patient complains of severe pains in the lumbar region but has no headache. Diarrhœa is present, the stool being watery in character. No appetite. His general appearence is typical of dengue.

August 5: Still complains of the lumbar pain; at 9 p. m. complained of abdominal pain, accompanied by slight nausea, vomiting and diarrhœa. No eruption has been observed.

August 6: Patient feels well.

August 7: Patient feels well.

August 8: Has headache and pain in muscles and joints. Last night was very nervous, almost delirious. Otherwise feels well.

August 9: Feeling well. Returned to duty August 14, 1906.

Remarks.—In this case the incubation period, as shown by the accompanying chart, was approximately two days and nineteen hours. The temperature curve is typical of a moderately severe case of dengue and the symptoms corresponded; the terminal rise and fall is well shown in this chart. No eruption occurred at any time, although otherwise the symptoms were typical, with the exception of the diarrhœa, which was present for a short time, accompanied by nausea and vomiting, which we regard as rather the exception than the rule in dengue.

Experiment No. 3.

Case 3, Chart 3.—E. W., private, first class, Hospital Corps, United States Army. At the time of inoculation this man was perfectly well and had not been exposed to dengue. He was given an intravenous inoculation at 2 p. m., August 16, of 20 minims of blood from Case 36 (see Chart C). The latter case was typical of dengue, which, at the time the blood was taken had lasted a little over three days. The clinical record of Case 3 follows:

August 16: Inoculated with dengue blood at 2 p. m. to-day, the injection being made into a vein of the forearm.

August 17 and 18: Patient feels well.

August 19: Slight temperature last night, but no headache, pain or other symptoms.

August 20: Last night patient suffered from headache, pain in bones and muscles of back, and severe pain in the knee articulations. Slept poorly. This morning has headache, located in the temporal region, and general muscular pain. Eyeballs are tender to the touch and also pain. Stomach and bowels normal. No appetite. An eruption, which appeared this morning covers almost the entire body, but is especially marked over the trunk and arms; it consists of very fine, slightly elevated papules, dark-red in color, the color disappearing upon pressure.

August 21: Feels somewhat better this morning. Has pain in eyes, shoulders, wrists and knees. The eruption is still present but is not so vivid in coloring as yesterday. Appetite poor. Bowels regular. Tongue clean and moist.

August 23: Patient had more headache last night but feels much better this morning. Is having very little pain now. The eruption is still present and the itching is constant. The bowels are loose. Appetite good.

August 24: Had headache last night which caused insomnia, also aching pain, severe in character, in muscles and joints. The eruption has greatly increased, covering the entire body, being especially marked over the trunk and limbs.

August 25: Patient slept well and all symptoms have disappeared except slight headache and pain in the eyes. The eruption is fading and the itching is very annoying; there is considerable desquamation present in the form of small white scales.

August 27: Eruption has disappeared. Patient feels well.

August 31: Returned to duty.

Remarks.—The incubation period in this case was about two days and eighteen hours. The temperature curve is not as characteristic as is generally observed, but the symptoms were very typical, and the fact that we were able to produce a very severe case of dengue by the injection of the filtered blood of this patient proves beyond doubt the nature of the disease (see Case 9, Chart 9). An interesting feature of this case is the early appearance of a well-marked eruption, which, after fading gradually, increased again during the crisis and finally disappeared, accompanied by considerable desquamation. It is also interesting to note that in the case of dengue referred to as being produced by the inoculation of filtered blood from this case, the eruption appeared early and presented the same characteristics. While the temperature was low the symptoms were more severe than in many others in which the fever was much greater, and the eruption was almost as well marked as in any case that we have observed.

In the three cases just described the inoculations were made as soon as the subject volunteered, no previous experiment in the way of exposure to fomites or mosquitoes having been tried. In the case which follows, the inoculations were used as a final test of immunity, the men inoculated having been exposed to both fomites and infected mosquitoes.

Experiment No. 4.

Case 4, Chart 4.—C. H. B., private, Troop A, Eighth Cavalry. Had been exposed to dengue, his troop having had eighteen men on sick report with the disease. At time of volunteering he was in good health, and stated that he had never had any serious illness. The following is a brief résumé of the clinical record in this case:

September 22: Exposed to fomites of dengue, being placed with three dengue cases in a mosquito-proof tent, sleeping in their beds and wearing their underclothes.

September 26: No result being obtained from fomites after four days, the patient is to sleep to-night under net with about forty mosquitoes which bit Case 38, who had a typical case of dengue (see Chart D) on the night of September 25.

September 27: Feels well. Says he was not bitten by a single mosquito.

September 28: Feels well. Says that he has not yet been bitten by the mosquitoes.

September 28 to October 2: During this time the patient has slept under the net containing the infected mosquitoes, but claims that he has not been bitten at all, and his statement is confirmed by the fact that the mosquitoes all remained empty during this time, and made no attempt to bite, most of them dying while within the mosquito net. He states that so far as he knows he has never been bitten by a mosquito, although he had campaigned in localities in Africa which were almost uninhabitable because of these insects. At first we were inclined to doubt his statements in this respect, but observation has convinced us that this man is really immune to mosquitoes.

October 3: At 10 a. m. the patient was given intravenously 20 minims of blood from Case 44 (see Chart E), a very typical case of dengue of about three and one-half days' duration.

October 4 and 5: Feels well.

October 6: Last night about 10.30 the patient complained of fever and muscular pains. This morning has severe pain in head, back and limbs. Face greatly flushed, conjunctivæ congested. Bowels constipated. Tongue moist with thin white coating.

October 7: Vomited last night. Has less pain this morning, located mostly in the muscles of the loins and thighs. Has considerable headache and pain in the eyes upon movement. Tongue moist and entirely covered with a yellowish coat. Bowels normal. Feels much better but still has muscular pain and headache.

October 9: Feeling well.

October 10: Had headache and pain last night, but feels well this morning.

October 15: Returned to duty.

Remarks.—The temperature curve in this case is one often observed in naturally acquired dengue and the symptoms throughout were typical of the disease. The absence of an eruption is to be noted, not that it is unusual in many natural infections, but because in our experimental cases an eruption was almost always present. Thus of the ten cases in which we were successful beyond doubt in producing dengue, eight presented well marked eruptions, while one was somewhat doubtful in this respect.

Experiment No. 5.

Case 5, Chart 5.—C. R. D., second-class private, Company B, Engineer Corps, United States Army. This man at the time of volunteering was in good health, but had been exposed to dengue during the Fort William McKinley epidemic. He was observed for a period of four days before any experiments were made. The following is the clinical record of this case:

September 19: At 1 p. m. a small abrasion was made on the mucous membrane of his cheek, and the patient then rinsed his mouth with blood from a dengue case, diluted with salt solution. No results were obtained from this experiment.

October 4: Patient slept last night under a mosquito-bar with mosquitoes that had bitten a typical case of dengue the night before. He was bitten several times during the next few nights, but dengue did not develop.

October 22: At 3 p. m. to-day an intravenous inoculation of 20 minims of blood from a dengue case (Case 60, Chart F) was given the subject.

October 23, 24, and 25: Patient is feeling well.

October 26: At 7 p. m. to-day the patient complained of headache, pain in the lumbar region and the legs, and loss of appetite.

October 27 and 28: Patient complains of severe headache, pain in the lumbar region and in the joints. His hands and wrists are slightly swollen, and the face, arms and hands greatly flushed. He is constipated and has no appetite.

October 29: Feeling much better. An eruption has appeared upon the chest and abdomen, resembling more the eruptions due to heat than a dengue eruption. He is still constipated and has but little appetite.

October 30 and 31: Patient feels well. There is a marked rash over the back and chest which upon the 31st had extended to the arms and legs. This rash is a typical dengue rash.

November 1: The rash is still well marked and is present over the entire body, including the palms of the hands and the soles of the feet.

November 2: Patient feeling well and the eruption has almost disappeared.

November 5: Returned to duty.

Remarks.—It will be observed that this patient did not contract dengue through the mucous membrane of the mouth nor from mosquito bites. As regards the latter experiment, we can not be sure that he was bitten more than one or two times by mosquitoes. From the intravenous inoculation of dengue blood he developed a very typical case of the disease as will be seen by referring to the temperature chart. The incubation period in this case was longer than in any of our previous cases, being four days and four hours.

Experiment No. 6.

Case 6, Chart 6.—J. E. S., private, Troop H, Eighth Cavalry. At time of experiments this man was in good health, but had been exposed to dengue at Fort McKinley. The following is a record of the experiments performed in this case:

September 22: Exposed last night to mosquitoes that bit Case 41 (Chart G) on September 13. Says that none bit him during the night.

September 23: Exposed again last night to mosquitoes, and says he was bitten once. Dengue did not result from this experiment.

October 7: Patient was exposed to mosquitoes that bit Case 44 (Chart E) the night before.

October 8 to 15: Patient feeling well, and states that he does not know whether he has been bitten. Dengue did not result from this experiment.

October 25: Slept last night with mosquitoes that had bitten Case 60 (Chart F), and was bitten at least twice.

October 26 to 28: Mosquitoes have disappeared and patient has not been bitten again. Dengue did not result from this experiment.

October 31: Slept with mosquitoes that had bitten Case 65 (Chart J) last night.

November 1 to 3: Has been bitten several times by the mosquitoes.

November 4: Patient feeling well and mosquitoes have all disappeared. Dengue did not result from this experiment.

November 8: At 3 p. m. to-day the patient was inoculated intravenously with 20 minims of blood from Case 70 (Chart K). The man from whom the blood was taken had a typical attack of dengue fever, and the inoculation was made upon the third day of the disease.

November 10 to 14: Patient feeling well.

November 15: In the afternoon the patient had a slight chill, headache and general pains in the muscles.

November 16: Patient complains of severe headache, backache and pain in the limbs. Has no appetite.

November 17 to 20: During this time the patient presented the usual symptoms of dengue, which have already been indicated.

Remarks.—The chief point of interest in this case is the long period of incubation, exceeding that of any experimental case that we have observed. Inoculation was made at 3 p. m. on November 8, and the first symptoms appeared in the afternoon of November 15, making the incubation period about seven days. It should be remembered that this man had already passed through a severe epidemic of dengue, and it is probable that he possessed a relative immunity to the disease, although his clinical symptoms were typical and rather severe in character.

Experiment No. 7.

Case 7, Chart 7.—W. J., private, Troop D, Eighth Cavalry. This case is of interest because the experimental dengue was complicated by an attack of malarial fever. He had been exposed to dengue at Fort McKinley but was in good health at the time of volunteering. The following is the clinical record of this case:

September 19 to 26: Patient was exposed to fomites of dengue during this time in the usual manner.

September 26: Exposed to mosquitoes that had bitten Case 80 (Chart L) the night before. This case was afterward found to be suffering from æstivo-autumnal malaria.

September 27 to October 8: Patient is feeling well.

October 8: Slept last night under a bar with mosquitoes that bit Case 4 (Chart 4), and upon the night of the 9th was bitten twice. Dengue did not result from this experiment.

October 25: Slept last night under a bar with mosquitoes that bit Case 81 (Chart M) the night before. Patient states that he did not feel well during the night, and complains of pain in the back of the head and in the lumbar region.

October 26 to October 28: Patient is feeling better. æstivo-autumnal malarial parasites were found in his blood on the afternoon of October 27, and quinine at once administered.

October 30 to November 4: Patient is feeling well. No further experiments were instituted until November 17.

November 17: Inoculated at 10.30 a. m. with 20 minims of blood from Case 82 (Chart N), who was suffering from a typical attack of dengue, which had lasted about three days.

November 18 to November 23: Patient feeling well.

November 24: At 10 a. m. patient had a slight chill, followed by high fever. Upon November 22 an eruption had been noticed covering the entire body, which resembled the eruption of dengue.

November 26: Patient is feeling well, and is free from pain. He states that he had had considerable pain for several days before his chill. He is covered with an abundant rash which presents all the characteristics observed in our other dengue cases.

Remarks.—There are several points of interest in this case. Upon September 26 he was exposed to the bites of mosquitoes that had bitten a case of æstivo-autumnal malaria. Upon October 25 he developed an attack of malarial fever, and the æstivo-autumnal parasites were found in his blood. He denied that he had ever suffered from malaria previously, and while we have been careful to liberate only mosquitoes of the genus *Culex fatigans* Wied., beneath the bars of the men experimented upon, it may be that an *Anopheles* may have been present with the mosquitoes liberated. However, as the *Anopheles* mosquitoes are present here, it is much more probable that the infection occurred from mosquitoes which had bitten a malarial case at some other time. It is difficult in this case to determine just exactly the period of incubation, and the chart is also atypical because of the concurrent malarial infection. However, the clinical symptoms, were very typical, and the presence of the rash removes all doubt as to the nature of the infection.

Experiment No. 8.

Case 8, Chart 8.—R. R., private, Company H, Thirteenth Infantry. This man was in good health at the time of volunteering, but had been exposed to dengue during the epidemic at Fort William McKinley. The following is a clinical record of the case:

September 12: Inoculated intravenously with one-half minim of blood from Case 83 (Chart O), who was suffering from a typical attack of dengue.

September 15: Patient states that he had a severe headache last night, commencing at midnight. Still complains of headache and pain in the arms.

September 16: Still complains of headache, but has no other pain. Bowels constipated, appetite poor.

September 17 to 19: Patient is feeling well.

September 19: Inoculated intravenously at 1 p. m. with 1 cubic centimeter of filtered blood from Case 11 (Chart 11). No result.

September 25: Inoculated intravenously at 1 p. m. with 20 minims of blood from Case 38 (Chart D). No result.

Remarks.—We have regarded this case as doubtful, although we are inclined to believe that the rise of temperature upon September 15 was due to a slight attack of dengue produced by the inoculation of one-half minim of dengue blood. This is much less blood than we have used in our other experiments and it may be that the slight symptoms produced are due to this fact. If this man did not suffer from an attack of dengue during his first inoculation he must have been immune, as neither the subcutaneous inoculation of filtered blood or the intravenous inoculation of unfiltered blood produced any result.

Summary.—The intravenous inoculation of unfiltered dengue blood into healthy men is capable of producing a typical dengue attack in such men. Thus, of eleven men so inoculated, seven suffered from dengue fever produced in this manner, while in one the result was doubtful. Three of the men were immune to the disease after inoculation.

5. INTRAVENOUS INOCULATION OF FILTERED DENGUE BLOOD.

Having proven by our inoculation experiments with unfiltered blood from dengue patients that the disease could be thus transmitted and, furthermore, that while the cause must, therefore, be present in the blood, it is not possible to demonstrate it in either fresh or stained specimens by any known method of examination, we are forced to the conclusion that the causative organism must be ultramicroscopic, as in the case of yellow fever, rinderpest, etc.

The most important diseases which have been proven to be due to a contagion which passes through porcelain or diatomaceous filters are foot-and-mouth disease, pleuro-pneumonia of cattle, yellow fever, rabies, rinderpest, South African horse sickness, and hog cholera.

Loeffler and Frosch (26), in 1898, discovered that the cause of foot-and-mouth disease in cattle passed through the pores of a Berkefeld filter which restrained other well-known bacteria; Nocard and Roux (27), in the same year, proved that the organism causing the pleuro-pneumonia of cattle passed through a Berkefeld and Chamberland F filter, but not through a Chamberland B; they were also successful in cultivating the organism by the collodion-sac method, but as they demonstrated that it could be seen, though indistinctly, with a power of 2,000 diameters, it can hardly be claimed that this organism is ultramicroscopic. In 1902, Reed and Carroll (28) filtered the blood of yellow fever patients and proved that the virus passes through a Berkefeld filter by producing the disease by inoculating the filtrate; Rosenau and Francis (29), in 1903, found that the cause of yellow fever passes through a Chamberland B filter. The virus of rabies, according to Remlinger and Riffat Bey (30), passes through the most porous of the Berkefeld filters, but not through other porcelain or diatomaceous filters. In rinderpest, Nicolle and Adil Bey (31) have proven that the cause passes through the Berkefeld and Chamberland F filters; Nocard (32), in 1901, found that the virus of South African horse sickness passed through the Berkefeld filters, and in 1900, McFadyean (33) demonstrated that it also passed through the pores of a Chamberland F and Chamberland B filter. The recent investigations of Dorset, Bolton, and McBryde (34) prove that the organism causing hog cholera is filterable through Berkefeld, Chamberland F and Chamberland B filters, and as easily through one as the other; they also prove that the organism so long regarded as the cause of the disease—that is, *B. cholera suis*—is not concerned in the etiology of hog cholera, the disease being produced by the filterable virus. Of the diseases mentioned, the causative organism has been demonstrated, with the highest power of the microscope, in but one—that is, pleuro-pneumonia—and in this instance no morphological details could be distinguished, the organism appearing simply as a minute motile point. In all the others the parasites are ultramicroscopic.

In order to determine if dengue belonged to this class of infections, we determined to try the effect of the intravenous inoculation of filtered blood from dengue patients into healthy men. We have experimented in this way upon two men in both of whom we have been successful in producing very typical attacks of dengue accompanied by rather severe symptoms.

FILTERS USED AND CONTROL METHODS.

In our filtration experiments we have employed a Lilliput diatomaceous filter, which was tested each time before it was used. Before using, the filter was sterilized and the filtration done under 730 millimeters pressure.

After filtering the blood the following control test of the filter was made in each case: A suspension, in nutrient bouillon, was made of *M. melitensis* and *S. choleræ,* and then filtered through the filter used in filtering the blood; the filtrate was then incubated for two weeks, daily examinations of it being made. The filter in use retained both these organisms, the filtrate remaining sterile for two weeks when it was thrown away. In the control filtrations the same filter was used, after careful sterilization, as was employed for the dengue blood, and the same pressure was maintained during filtration.

Besides the control test of the filter, we kept in each case a portion of the filtered dengue blood for a period of ten days, making daily examinations, and in one case, several cultures in bouillon. No growth was obtained in either the filtered blood or the cultures.

Experiment No. 9.

Case 9, Chart 9.—E. J. D., private Hospital Corps, United States Army. On August 21, 1906, 10 cubic centimeters of blood was drawn from the median basilic vein of Case 2, an experimental case of dengue which has been described (see Case 2, Chart 2). The blood was taken on the third day of the disease, the symptoms of the patient at the time consisting of fever, headache, severe pain in the muscles of the shoulders, in the wrists and knees, while there was present a typical dengue eruption.

The blood was rapidly defibrinated, diluted with an equal amount of normal salt solution, and filtered through a Lilliput filter, controlled as described. The filtration of a sufficient quantity for use was completed in about three-quarters of an hour. Of the filtrate, 50 minims, containing 20 minims of the filtered blood, was inoculated intravenously into Case 9 at 4.20 p. m., August 21. The patient at the time of inoculation had been in the hospital under observation for several weeks, and as no cases of dengue had occurred in the hospital, he had not been exposed to the disease. No symptoms of importance developed until August 25, the period of incubation being three days and eleven hours. Previous to the decided onset of the disease there had been slight fever and some pain in the back, symptoms which were probably due to a gonorrhœa from which he had suffered for some time. The following is the clinical record of this case:

August 21: Inoculated intravenously at 4.20 p. m. with 25 minims of filtered dengue blood from Case 2.

August 22 and 23: Feeling well.

August 24: Slight muscular pains.

August 25, 9 a. m.: Patient complains of pain in muscles of neck, shoulders and knees. Has some headache. Bowels constipated. Tongue moist and clean.

August 25, 4 p. m.: The symptoms have increased in severity. The headache is frontal and intense; there is severe pain located behind the eyeballs, which are painful on pressure. There is general muscular pain, especially in the muscles of the jaw, lumbar region, and in the calves of the leg. Patient states that his bones ache and that he is unable to rest comfortably in any position. He also complains of severe pain in the articulations. An eruption is present, covering the chest, abdomen and thighs, and is especially marked over the forearms and around the wrists; it is dull red in color, consisting of minute elevations surrounded by a vivid flush, which makes the rash appear confluent in character.

August 26: Patient passed a very restless night, suffering from insomnia and severe pain in the back, chest, legs, head, and eyes. This morning still has severe pain in these regions. There has been no vomiting and the bowels are constipated. Tongue is moist, with a slight white coating. The eruption covers the entire body and is more marked than yesterday. There is complete loss of appetite and the patient is very restless.

August 27: Feels much more comfortable this morning. The steady ache in the muscles has disappeared but he still suffers from lancinating pains in the head, back, and legs. The eruption has almost disappeared.

August 28: Passed another restless night and suffered a great deal from pain in the muscles. This morning he complains of severe headache and pain in the loins and legs. The eruption has faded from the trunk. Has no appetite.

August 29: Feels better this morning. Still has headache and pain in the eyes, but the general muscular pain has disappeared.

August 30: Still complains of pain in the head and eyes but slept well last night.

August 31: Patient states that he feels very well this morning. Has no pain and appetite is returning. There is present a very profuse dengue eruption covering the entire body, especially marked upon the arms, legs, hands and feet.

September 1: Feels well. The eruption is less distinct although it still covers the entire body.

September 2: Is feeling well in every away and eruption has disappeared.

September 4: Discharged.

Remarks.—This case, as shown by the clinical record and the chart, is typical of severe dengue, but the initial eruption was more marked than in any of our cases. The patient suffered greatly from the headache and the muscular pains. He repeatedly stated that he felt as though every bone in his body had been broken.

The temperature chart presents a high range of fever, with not as marked a period of remission as is generally observed; it will be noted that morning remissions occurred regularly, but that in the afternoon the temperature ascended, reaching 104° F. on three consecutive days; a more permanent remission occurred upon the fifth day, succeeded upon the sixth by the final rise and the crisis, the temperature reaching normal upon the seventh day.

An eruption appeared in this case upon the first day (the so-called initial eruption), extending over the chest, abdomen and thighs. The typical dengue eruption occurred, as is usual, during the crisis, and was very profuse, extending over the entire body, even the hands and feet being covered with it. The severe initial eruption in this case is very unusual, and it is most interesting to find, upon reference to the clinical history of Case 2, from whom this man was inoculated, that an eruption occurred in this case also upon the second day of the disease.

Experiment No. 10.

Case 10, Chart 10.—B. S., first-class private, Hospital Corps, United States Army. On August 31, 1906, at 12.15 p. m., this man, who had been on duty at the Division Hospital for weeks and had not been exposed to dengue, was given an intravenous injection in the arm of 3.75 cubic centimeters of normal salt solution containing 20 minims of dengue blood from Case 87 (Chart H). Ten cubic centimeters of blood was taken from the medium basilic vein of Case 87 at 10.30 a. m., August 31, diluted with normal salt solution, and filtered through the same filters used in Case 9, the filter being controlled as has been described. This filtered blood was used for the inoculation. The patient from whom the blood was obtained was suffering from a rather severe attack of dengue, and the blood was taken on the fourth day of the disease.

After inoculation with the filtered blood no symptoms appeared in Case 10 until midnight of September 3, but upon referring to the temperate chart it will be noticed that he had fever at least sixteen hours before he complained of any symptoms. If we assume that the first rise in temperature indicated the onset of dengue, the incubation period must have been about two and one-half days, while if the chill, which was the first symptom the patient noticed, is considered as marking the onset, the incubation period would be just three days. We consider that the incubation period in this case was two and one-half days. The following is a résumé of the clinical record of this case:

August 31: Inoculated intravenously at 12.30 p. m. with 20 minims of filtered blood from Case 87.

September 1: Was restless last night but at noon to-day feels well.

September 2 and 3: Feeling well.

September 4: Had chill last night about midnight. This morning complains of pain in the muscles and bones, especially of the arms. His eyes ache and are much congested and the face is flushed. Has slight frontal headache, and is very nervous. He complains of palpitation of the heart. Tongue moist and clean. Appetite poor.

September 5: Is feeling very nervous this morning and was delirious last night. Has pain in head, back, arms and legs. No appetite. Tongue moist, with heavy, yellowish coating. Bowels loose.

September 6: Spent a restless night, but is not so nervous this morning. Complains of severe pain in the back and legs. Tongue moist, with yellowish coating. There is a faint, slightly elevated, sparse, macular eruption over the chest and back.

September 7: Patient had a comfortable night and this morning has but little pain. The eruption is well marked over the abdomen, chest, back and arms.

September 8: Feeling very comfortable. The eruption is fading a little. Bowels constipated. Appetite good.

September 9: Was delirious during the early morning hours and is nervous and restless this morning, but free from pain. The eruption has largely disappeared.

September 10: Began to feel better at 4 p. m. yesterday and now feels quite well. Slept well, but perspired very freely during the night. The eruption has almost disappeared from the body, but is marked upon the forearms and wrists.

September 11: Feeling well. Eruption is fading slowly and very slight desquamation is present in patches.

September 13: Feeling well, except that appetite is still poor. The eruption has almost disappeared.

September 15: Returned to duty.

Remarks.—The symptoms in this case were very severe, especially those connected with the nervous system. The subject of the experiment was of a highly nervous temperament, and this fact accounts, in our opinion, for the severity of the nervous symptoms.

The temperature curve in this case might be used as an illustration of an ideal dengue curve, so perfectly does it agree with the type described by every observer as characteristic of this disease. It should be noted, however, that the temperature is higher in this case than it usually is in naturally acquired dengue, or in our other experimental cases, with the exception of Case 9, also produced by the intravenous inoculation of filtered blood.

The eruption in this case appeared on the fourth day of the disease, and had disappeared on the third day following the crisis, lasting in all ten days.

We regard these two cases of dengue produced by the intravenous injection of filtered dengue blood as the most typical cases of the severe type of the disease which we have observed and we believe that these two experiments prove conclusively that dengue can be transmitted by blood which has been passed through a filter which retains organisms as small as 0.4 μ in diameter (the measurement of *M. melitensis*). It also proves that in all probability the causative agent is ultramicroscopic in size, for the reason that neither in fresh nor stained blood smears nor in the filtrate obtained from dengue blood, can any organism be demonstrated with the microscope. It may be possible that in some other fluid or organ of the body, or in some phase of its life history in an insect, the organism may be visible, for Novy, in his work upon *T. lewisi,* has proved that even so large a parasite as this trypanosoma may exist in a form so small in cultures that it passes through a Berkefeld filter. While this may prove to be true as regards the dengue organism, we feel justified in stating, that, so far as present evidence goes, the organism causing dengue is ultramicroscopic in size. This conclusion explains the uniformly negative results obtained by nearly every trained observer in the search for a dengue parasite.

We conclude that an organism is present in the filtrate, rather than a toxin, because of the length of the period intervening between inoculation and the appearance of clinical symptoms, and also because we have reproduced the disease by inoculation of the blood of experimental cases.

There is one point of interest deserving of special consideration in these two cases of dengue produced by filtered blood; that is, the relatively greater severity of the symptoms. In both these cases the symptoms were more intense in almost every particular than in any of our experimental cases, despite the fact that no greater amount of blood was inoculated in these cases. This fact is very difficult of explanation, and we must confess to our ignorance of the cause. It may be that the admixture with salt solution or the time consumed in filtration, or both, acts in some way to increase the virulence of the organism, or that conditions favorable to its extra-corporeal development are present during the process of preparing the filtrate which result in a more virulent form of the organism, though we have no evidence to offer in this respect.

6. EXPERIMENTAL TRANSMISSION OF DENGUE BY THE MOSQUITO.

We have already mentioned the experiments of Graham (21), regarding the transmission of dengue by the mosquito, in which he seems to have proven conclusively that such transmission occurs; we have also noted the negative results obtained by Carpenter and Sutton (22), Guiteras and Cartaya (7), and Agramonte (19), all of whom believe, however, that the mosquito is the active agent in the spread of the disease. To one who carefully studies the epidemiology of dengue, the conclusion is almost inevitable that this disease, which so closely resembles yellow fever and malaria in this respect, must also be transmitted by some species of mosquito. Its seasonal prevalence; its occurrence most frequently along low-lying, moist, coast regions and in the valleys of rivers; its rapid diffusion in certain localities, and its lack of diffusion in others; its relation to changes in temperature and moisture; its manner of spread from building to building in infected places; the presence of multitudes of mosquitoes wherever dengue occurs, and the absence of the disease in regions where mosquitoes are few in number or absent, and the cessation of the epidemic in badly infected districts when conditions arise unfavorable to the propagation of mosquitoes, all point to some species of this insect as the transmitting agent.

Accordingly, having demonstrated by the intravenous inoculation of unfiltered dengue blood that the cause of the disease is present in the blood of the infected individual, and that the parasite is probably ultramicroscopic in size, as proven by the positive results of our experiments with the filtered blood, we turned our attention to the problem of mosquito transmission. Unfortunately for the fullest success of our work in this direction, we were forced because of lack of other volunteers, to use a number of men who had already passed unharmed through the epidemic at Fort McKinley and the majority of whom were immune, as is proven by the negative result of intravenous inoculation of dengue blood. Thus, of the nine men in whom we endeavored to produce dengue by exposing them to the bites of infected mosquitoes, three were proven in this way to be absolutely immune, one may have had a slight attack of dengue previous to exposure, while three probably possessed a relative immunity, for while they developed dengue from the inoculation of a comparatively large amount of dengue blood, the symptoms were mild in character, and in one case the incubation period was greatly prolonged. In one instance already described (see Case 4) no immunity existed to the disease, but the mosquitoes refused to bite the man under any conditions we could devise.

The mosquito used.—In looking over the geographical distribution of dengue and various species of mosquitoes, we found but one species (*Culex fatigans* Wied.,) of this insect that apparently occurred wherever dengue did. We do not wish to be understood as stating conclusively that this mosquito is the only one which may be present in all dengue-infected regions, but only that, so far as we have been able to determine from the literature available, this species is constantly found and is mentioned by almost every recent investigator as being very numerous during epidemics of this disease. In Theobald's monograph the map illustrating the known distribution of *Culex fatigans* Wied. might almost be used to illustrate the distribution of dengue fever, and if to this map be added the regions in which this mosquito has been demonstrated since it was published, the association of dengue and *Culex fatigans* Wied., is still more striking.

For this reason, and because this mosquito was employed by Graham in his experiments, we decided to work with this species at first, and in the event of our results being negative, to extend our work to embrace other species.

We have used mosquitoes reared in captivity, and also those caught in natural surroundings. However, in our successful case produced by the mosquito, we used mosquitoes reared by us from the egg, and thus we are sure that no infection occurred in these insects before they bit the dengue patients.

Our mosquito experiments were conducted as follows: The patient suffering from dengue was placed in a bed beneath a mosquito net in a mosquito-proof tent. At night from twenty to thirty mosquitoes were liberated beneath the mosquito bar and collected in the morning; almost invariably all the mosquitoes left alive had bitten and were full of blood. The subject to be experimented upon, having been placed in bed beneath a mosquito net in another mosquito-proof tent, the mosquitoes which had bitten the dengue case the night before were liberated beneath his mosquito net, and orders given that the man remain beneath the net until the mosquitoes had disappeared; later we allowed the men to remain out of bed during the day, the mosquitoes being kept beneath the spread net. With one exception, which has been noted, all the men were bitten a few times, but in most instances the mosquitoes died before the men had been bitten severely. We also confined mosquitoes that had bitten dengue cases in glass jars, and kept them as long as from four to six days before allowing them to bite, but in the few instances in which we tried this method our results were all negative.

We do not consider it necessary to give our negative results in full, as they are all referred to in detailed experiments, and we will only describe the case in which we produced dengue by allowing the volunteer to be bitten by infected mosquitoes.

Experiment No. 11.

Case 11, Chart 11.—B. L. W., private, Hospital Corps, United States Army. This man had been on duty at the Division Hospital for several weeks, and as no cases of dengue had occurred in the hospital, had not been exposed to the disease, so far as we could determine. On September 12, 1906, the man being in good health, he was placed under a mosquito net with mosquitoes that had bitten Case 88 (Chart R) on the night of September 11. Case 88 was suffering at the time from a typical attack of dengue. Case 11 was not bitten by mosquitoes until the night of September 13, and developed no symptoms until the night of the 17th, but upon reference to his chart it will be seen that he had fever for nearly twenty-four hours before he noticed any symptoms. If we assume the period of incubation to be the period intervening between the 13th, the night upon which he was first bitten, and the 16th, when he had his first rise in temperature, the incubation period would be about three days and one-half. However, if we assume the disease to have commenced when he first noticed symptoms—that is, upon the evening of the 17th—the incubation period would be a little over four days. The following is a summary of his clinical history:

September 12: Put under net with mosquitoes that bit Case 88 last night.

September 13: Bitten by mosquitoes last night.

September 18: Had headache and felt uncomfortable last evening. This morning complains of headache and a dull pain in the articulations.

September 19: Still complains of headache and general muscular pain and soreness. His face and eyes are greatly congested.

September 20: Last night had severe pain in the head, eyes and the muscles of the back, but feels much better this morning.

September 21: Is feeling better. A faint rash is visible covering the chest and abdomen.

September 22: Complains of soreness and stiffness in the muscles. The eruption is now plainly visible and typical of dengue.

September 24: Feels well. The eruption has almost disappeared.

October 1: Returned to duty.

Remarks.—This case was in every way typical of a moderately severe attack of dengue. The symptoms were those seen in the great majority of naturally acquired infections and the temperature chart is a very characteristic one. This man had not been exposed in our dengue camp before being bitten by the mosquitoes, and did not leave the mosquito-proof camp until after the onset of the disease.

For reasons which have been stated, of the nine men exposed to the bites of infected mosquitoes, only four can be considered in estimating the results obtained. Of these, one, or 25 per cent, developed a typical attack of dengue following the bites of infected mosquitoes; but we do not consider that the three negative cases are of much value, as the conditions were such as to cause some doubt as to whether the men were bitten.

It is obvious that many factors have to be considered in considering mosquito experiments, and it is more than probable that in our negative experiments we were unsuccessful in reproducing the favorable conditions which must have been present in Experiment No. 11, or the mosquitoes, if they became infected, may have perished before biting again. Schaudinn has recently called attention to some of the difficulties which may be met with in attempting the experimental transmission of a disease by mosquitoes. Thus, as he has shown, certain individuals of a species which has been proven to transmit a certain disease are not able to transmit it, and this may be due to the insect itself suffering from some other infection, to an inability to digest the ingested blood, to an acquired or natural immunity resulting in the death of the specific parasite, or the mosquito may die before it has bitten again.

It is evident from the result of Experiment No. 11 that the parasite causing dengue does not undergo any cycle of development within the mosquito, unless it be a very short one; we are, therefore, of the belief that the parasite of dengue is one capable of living in the stomach of the mosquito for an unknown period of time, where it retains its virulence; that infection may occur at any time after the insect has ingested blood containing the parasite, and that it is introduced into man when the insect bites, being regurgitated through the œsophagus and proboscis with the fluid from the stomach. This theory is borne out by the results recently obtained by the Indian Plague Commission in its remarkable study of the transmission of plague from rat to rat by the flea, *Pulex cheopis* Rothsch., and by the excessive rapidity of the diffusion of dengue, which would be impossible were the parasite one which underwent a prolonged cycle of development in the tissues of the mosquito. We have dissected and examined a large number of mosquitoes that had bitten dengue patients, but have never found any organism either in the stomach or tissues suggestive of a stage in the life cycle of a protozoön. We can not confirm Graham's results in this respect, and we believe that in the mosquito as well as in the blood of man, the dengue parasite is ultramicroscopic in size.

By reason of lack of suitable volunteers and the subsidence of the epidemic we have been forced to bring our mosquito experiments to a conclusion. We have been unable to investigate many interesting questions regarding the transmission of dengue by the mosquito, such as the length of time the insect remains capable of transferring the infection, the most infective period of the disease as regards transmission in this way, and whether transmission is simply mechanical or depends upon the development or multiplication of the parasite within the mosquito; all of these questions are of great importance to a correct conception of the etiology of dengue and there would appear to be no good reason why, in regions where the disease is common, they should not be thoroughly investigated. We realize that the work we have been able to do as regards mosquito transmission is very incomplete and that much remains to be done before this feature of the etiology of dengue is fully elucidated, but we believe that we have confirmed Graham's results in this respect and that we have proven experimentally that this disease may be transmitted by the mosquito *Culex fatigans* Wied. We also believe that mosquito transmission is the only natural method which has been proved by experiment and that all the epidemiological data confirm such a method of transmission.

7. EXPERIMENTAL PERIOD OF INCUBATION IN DENGUE.

As will be seen from a résumé of the epidemiology of dengue, the incubation period has been stated as varying from twenty-four hours to ten days, the majority of observers regarding it to be from three to five days. The following table gives the period of incubation in nine of our experimental cases of the diseases:

No. of case.	How produced.	Incubation period.
1	Inoculation of unfiltered blood	3 days 18 hours.
2	do	2 days 19 hours.
3	do	2 days 18 hours.
4	do	2 days 12 hours.
5	do	4 days 4 hours.
6	do	7 days
9	Inoculation of filtered blood	3 days 11 hours.
10	do	2 days 12 hours.
11	By mosquito	About 3 days 16 hours.

From the above table it will be seen that the incubation period of dengue in experimental cases of the disease varied from two and one-half days to seven days, the average being about three days and fourteen hours. This is practically the period of incubation stated as being most frequent by clinicians. We have observed no case in which the incubation period was as short as twenty-four hours, and from our experiments we very much doubt the occurrence of such a short period of incubation.

8. IMMUNITY AND SUSCEPTIBILITY.

There is considerable confusion existing in regard to these points, the general trend of opinion being that almost everybody is susceptible, and that an attack of dengue produces immunity for a short time only. As to the latter point—that is, the duration of acquired immunity—we can not express a very positive opinion, as we endeavored, except in one case, in our experiments to avoid the use of men who had previously had dengue. In the one case noted as an exception, dengue was induced although the patient said he had experienced three attacks, the last one two and a half years ago. We have also known a few other cases in which the disease developed naturally after a like period. The correctness of reports of cases in which attacks have occurred a month apart we very much doubt. We had about six patients sent back to us after such periods supposed to be suffering from second attacks, but in no case was it so. The "second attack" was usually a malarial paroxysm.

As to natural immunity, we know that it occurs, or at all events that it may be temporarily present. We think it altogether probable that it may be relative; that is, a small dose of virus may not be sufficient to overcome it, but a large one may. In one of our cases (Case 8) we were unable to decide positively whether an immunity which was present at the time of the discharge of the patient was natural or was acquired from a very light attack of the disease following inoculation with a half minim of blood, though we incline to the latter belief. In at least one instance immunity was apparent and not real; that is, the patient did not develop dengue when exposed to mosquitoes that had recently bitten other dengue cases, but this was due to the fact that the subject was immune to mosquito bites. All the mosquitoes put in his net died after periods varying from one to five or six days, and not one of them bit him. Later, when he developed dengue from the intravenous injection of blood, mosquitoes bit him freely. Fortunately, this characteristic so valuable in the Tropics, was not permanently lost, for the patient now states that he is as free from mosquito bites as before.

Our knowledge as to natural immunity cost us rather dear, as we were paying all our subjects of experiment, and did not relish exhausting the funds at our disposal in payments to men not capable of developing the disease. In the light of subsequent events we think that we made a mistake in accepting volunteers from Fort McKinley, where an epidemic of dengue had been and was prevailing; because, while we did not begin experiments upon the men until they had been under our observation and free from exposure to dengue for periods varying from a week to three weeks, and thus avoided the error of thinking the disease due to our inoculations when it was in reality due to other causes, we picked men, some of whom had probably escaped natural infection because of their natural immunity.

As we have stated, three of our subjects were absolutely immune to dengue. Our assumption, that failure to develop the disease after inoculation with 20 minims of blood from a dengue case constitutes absolute immunity, is arbitrary, but seems justified by the constancy and severity of the symptoms produced in the successful cases.

Three of the men possibly showed a relative immunity; that is, the amount of virus transferred to them by mosquitoes was not sufficient to cause the disease, although the intravenous injection of 20 minims of dengue blood was sufficient to do so. Possibly this relative immunity was only apparent, because we know that these men were not severely bitten by the mosquitoes, and we do not know that the particular mosquitoes that did bite them might not have been laboring under some disability that prevented their transmitting the disease. It is noteworthy that two of these cases were very mild, and that the third, while an ordinary one, presented an incubation period longer than the average.

Six cases, and if we count the doubtful one already described, seven, presented no immunity; that is, they developed dengue following the first attempt at inoculation. One case, immune to mosquito bites, showed apparent immunity, but developed dengue after the first inoculation.

Natural immunity and the practice of sleeping under mosquito nets effectually protected a large proportion of healthy men against infection. Thus, in the Fort McKinley epidemic, the highest percentage of infections occurring in any one company was 58, the next highest was 52, and in the other companies it was lower. It must be remembered that in this epidemic no special measures were adopted to prevent the spread of the disease, and the mosquito protection consisted merely of the ordinary routine use of nets during the sleeping hours.

IMMUNITY AS SHOWN BY EXPERIMENTS.

The following cases whose clinical records are here given were proved by experiment to be absolutely immune to dengue. The temperature charts are not reproduced, as they contain no data of interest.

Case 12.—W. H. O., first-class private, Hospital Corps, United States Army. This man was on duty at the Division Hospital at the time of experiments, and had never had dengue.

Experiment 1: On the night of September 12 the subject slept under a mosquito net with mosquitoes that had bitten a dengue case the night before. No symptoms of dengue developed.

Experiment 2: On the night of September 28 the subject was again exposed to mosquitoes that had bitten a dengue patient the night before. He was bitten repeatedly during the next few nights, but no symptoms of dengue developed.

Experiment 3: On October 3 the subject was inoculated intravenously with unfiltered dengue blood from Case 44 (Chart E). No symptoms of dengue developed, and the man was returned to duty October 11, 1906.

Case 13.—J. G., private, Company I, Sixteenth Infantry. This soldier belonged to a company of the Sixteenth Infantry that had furnished twelve cases to the hospital with dengue before this man volunteered. He had therefore been exposed to the disease.

Experiment 1: On September 12, 1906, the subject rinsed his mouth with normal salt solution containing 12 minims of dengue blood, our object being to determine if the dengue parasite could infect through an intact mucous membrance. The result of the experiment was negative.

Experiment 2: On September 19 the subject was given an intravenous inoculation of filtered blood from Case 11 (Chart 11). No symptoms developed.

Experiment 3: On the night of October 4 the subject was exposed to mosquitoes that had bitten Case 44 (Chart E) the night before, and was bitten at least twice. Dengue did not develop.

Experiment 4: On the night of October 15 he was bitten many times by mosquitoes that had bitten a dengue case two nights before. The result was negative.

Experiment 5: On October 22 the subject was given an intravenous inoculation of 20 minims of unfiltered blood from Case 95 (Chart S). No symptoms of dengue developed, and the man was returned to duty October 29, 1906.

Case 14.—J. B. P., private, Company M, Sixteenth Infantry. At the time of volunteering the company to which this man belonged had furnished ten men suffering from dengue to the Forth McKinley Hospital.

Experiment 1: On the night of September 24, 1906, the subject was exposed to mosquitoes that had bitten Case 11 (Chart 11) the night before. He was bitten several times and also many times during the next ten days. The result of the experiment was negative.

Experiment 2: The subject was exposed October 26 and 27 to mosquitoes that had bitten a typical case of dengue on October 25. The result of the experiment was negative.

Experiment 3: On November 17 the subject was given an intravenous inoculation of unfiltered blood from Case 82 (Chart N), who was suffering from a typical attack of dengue. No successful result was obtained in this experiment, and the man was returned to duty November 23, 1906.

Remarks.—These men were all exposed to fomites, in addition to the experiments outlined, and we believe that the results of these experiments demonstrate that absolute immunity to dengue is present in certain individuals.

9. CONTAGION IN DENGUE.

We have already noted the theories regarding the contagious character of dengue. We have carefully studied this portion of our subject, and believe that the following facts conclusively prove that dengue is not contagious in the least degree.

1. At the hospital at Fort William McKinley over 600 cases of dengue were treated in the general wards without a single case originating among the other patients in the wards. Only four men belonging to the Hospital Corps on duty at this hospital contracted the disease, three of them being nurses on night duty in the wards and the other a cook having no contact with the dengue patients. No precautions were used to prevent contagion other than the rigid use of mosquito nets at night, the dengue and other patients eating together, and being closely associated

throughout the day. It is noteworthy that the only men unprotected by the mosquito nets at night—that is, the three night nurses—all developed the disease.

2. In our dengue hospital, where we treated over 120 cases, no instance of infection occurred among the attendants, although their association with the dengue patients was very intimate and continued for over four months.

3. Our experiments with fomites were all negative. We endeavored to produce the disease by exposure of healthy men to fomites, the men experimented with living in mosquito-proof tents with patients suffering from dengue, throughout the entire course of the disease. They slept in their beds, wore their underclothing and pajamas, and ate and drank from the same table furniture. In this way we experimented with eight men, none of whom developed the disease from such exposure.

We conclude, therefore, that dengue is not a contagious disease, and that patients suffering from it may be placed in the general wards of a hospital without fear of infection, provided precautions are taken to protect the patients from mosquitoes.

Conclusions regarding the etiology of dengue.—From our study of the etiology of dengue, we believe the following conclusions are justified:

1. No organism, either bacterium or protozoön, can be demonstrated in either fresh or stained specimens of dengue blood with the microscope.

2. The red blood count in dengue is normal.

3. There occur no characteristic morphological changes in the red or white blood corpuscles in this disease.

4. Dengue is characterized by a well-marked leucopenia, the polymorphonuclear leucocytes being decreased, as a rule, while there is a marked increase in the small lymphocytes.

5. The intravenous inoculation of unfiltered dengue blood into healthy men is followed by a typical attack of the disease.

6. The intravenous inoculation of filtered dengue blood into healthy men is followed by a typical attack of the disease.

7. The cause of the disease is, therefore, probably ultramicroscopic.

8. Dengue can be transmitted by the mosquito, *Culex fatigans* Wied., and this is probably the most common method of transmission.

9. No organism of etiological significance occurred in bouillon or citrated blood cultures.

10. The period of incubation in experimental dengue averages three days and fourteen hours.

11. Certain individuals are absolutely immune to dengue, as proven by our experiments.

12. Dengue is not a contagious disease, but is infectious in the same manner as is yellow fever and malaria.

IV. SYMPTOMATOLOGY.

It is of cardinal importance in considering the symptoms and diagnosis of dengue to bear in mind the fact that it presents, in different epidemics and in different individuals in the same epidemic, a variety of clinical pictures; and that, while there is what may be called typical dengue, there are many variations from the type, and there is no one symptom that can be said to be pathognomonic, or even constant, if we except fever. We do not state positively that even fever is constant, but we are unable to satisfy ourselves that a given case is dengue unless it shows some fever, particularly at the onset. This doubtless accounts for the different descriptions of the disease that have been written. We agree with Guiteras and Cartaya in the belief that many cases can not be properly diagnosed except in the presence of an epidemic. We likewise agree with them that it is illogical to differentiate subtypes of the disease according to the dominant symptom, so we shall content ourselves with outlining the typical attack, and commenting on the usual symptoms. In doing this we will use the plan of the writers mentioned, whose observations and descriptions we consider accurate, clear and well balanced.

Invasion.—This is usually rather sudden, and, exceptionally in our experience, may be so sudden that the patient has to sit or lie down, being unable to continue the employment in which he is engaged. One patient was a sentry on post at the time he was attacked, and so sudden and severe was the onset that he had to call for relief. However, many cases have a gradual onset, and it was not uncommon for men to report sick with a history of having felt ill for a day or two, or even three. The onset is usually manifested by pain in the loins, often also in the legs, with headache and fever. Frequently the sensation is one of extreme weariness, rather than of pain. Chilliness is at times, but not usually, complained of. The appetite is nearly always impaired, and vomiting or diarrhœa are occasional features.

Catarrhal symptoms, such as coryza or bronchitis, are not present, unless as a complication, and are usually due to preëxisting causes. Sore throat is described as common in some epidemics. We observed it in very few cases, and consider it rare. The skin is usually much injected, especially over the head and neck. Injection of the conjunctiva and lachrymation are common signs; photophobia is uncommon. We have not seen jaundice of either skin or mucous membranes. The early injection of the skin is described by some authors as the primary eruption. We agree with Guiteras and Cartaya in thinking that this term should not be applied to it. There is, in practically all cases, but one eruption, and it appears later, if at all. We have seen one case in which two eruptions appeared, but it was the rare exception which only served to emphasize the rule.

In a few cases the onset is so gradual and its manifestations so mild that it may not be noticed at all. Case 8 of our experimental series, who also had an æstivo-autumnal malarial infection, is a case in point. The incubation period in this case and the date of the eruption indicate that he had had dengue for about four days, while a blood examination showed that his chill and high fever of November 25 were of malarial origin.

Fever.—Fever is in practically all cases present from the beginning, and in the majority it reaches its maximum within twenty-four hours. This primary rise may exceptionally be to 40.5° C. (105° F.), or even 41° C. (106° F.), usually it reaches to about 39.7° C. (103.5° F.). In a minority of cases the ascent is gradual (see Case 2).

By the end of twenty-four hours the temperature has usually fallen 1° C. (2° F.) or more, and the period of intermission has begun. In some cases this drop in temperature is delayed until the beginning of the third day, quite exceptionally the same high point may be reached on four or five successive days (see Case 9).

However, in the typical case the temperature has fallen as stated at the end of twenty-four hours. The fall may carry it to normal, or only as low as 37.8° C. (100° F.), 38.3° C. (101° F.), or 38.9° C. (102° F.). There it remains, usually until the fifth day, when it again rises to almost as high a point as its early maximum. On the sixth day there is generally a sudden fall, by crisis, to normal, and the disease is ended. Critical discharges do not, in our observation, usually attend this fall in temperature, though profuse perspiration may occur.

When the chart is "typical" it is very characteristic of the disease, and enables one to pronounce a correct diagnosis at sight. Often it is not typical. The sharp rise on the first day and another on the fifth or sixth day, occur sufficiently often, however, to make the temperature chart at least as characteristic as in many other diseases in which much diagnostic significance is attached to it, as in typhoid fever.

Guiteras and Cartaya (7) protest against the description of the disease as one characterized by two febrile paroxysms, and contend that the fever is one attack, usually lasting six days, and only exceptionally subsides to normal before the sixth day. We agree with them in this, though we see no more objection to speaking of two paroxysms in this disease, when the temperature does go to normal between them, than in speaking of paroxysms in malaria under similar conditions.

The variations of this "typical" temperature record are manifold, as is shown in very many charts in our possession. However, in the majority of instances the type may be recognized even through the variations.

Hyperpyrexia, causing dangerous symptoms, is mentioned as a rare occurrence. We have not observed it.

Meningeal symptoms may, according to Guiteras and Cartaya, so alter the chart as to make it unrecognizable. We have not seen such cases. Our most severe case, and the one in which we observed the most marked nervous symptoms, showed an almost "typical" chart (see Case 10).

Pulse.—The resemblance between beginning dengue and beginning yellow fever, and the dissociation of pulse and fever in the latter disease, give to the pulse of dengue an importance it would not otherwise merit. Guiteras and Cartaya, who studied and wrote of the disease with its differential diagnosis from yellow fever as their main theme, summarize their observations on the pulse by saying that "in general it is not slow as in yellow fever, and especially not in the first days, but that dengue shows a tendency to slow pulse."

We have seen in no case a markedly slow pulse, and think that in general the pulse follows the temperature fairly well, although the tendency to slowness is most apt to be manifested by a relatively small rise in pulse rate. Writing with little experience with yellow fever, we should consider the pulse a valuable diagnostic feature.

Pain.—Pain is usually described as the earliest symptom. This is true in nearly all cases, so far as the patient knows, but as before stated, it is often preceded for several hours by a rising temperature. The pain is frequently severe, infrequently excruciating and immediately disabling. Also in a few instances it is trifling and very rarely it may be absent. It is in nearly every case manifested as headache and almost as frequently as lumbar pain. In a smaller number, but still a large majority of all cases, it is also present in the limbs, especially in the calves of the legs; less often, but still not rarely there is abdominal pain.

The headache may be frontal, vertical, temporal, occipital or post-orbital. Of these varieties we should place frontal headache as first in order of frequency, post-orbital second, temporal third, and vertical and occipital as least frequent. Movement of the eyeballs is often a cause of pain, particularly in patients complaining of post-orbital pain.

The pains in the lumbar region, trunk and limbs are of varying severity, in many cases giving rise to most bitter complaint, in others only being mentioned in response to inquiry. Such inquiry will in the vast majority of instances, practically all, elicit an account of pain. This is described by Guiteras and Cartaya as being localized in the deep insertions of the muscles. This seems to be the condition at times, but almost as frequently the bodies of the muscles are affected, especially of those in the legs, where the fleshy calf is often very painful. In spite of the fact that the disease is called "break-bone fever," we have seldom had patients complain of pains in the bones.

Joint pains are not infrequently complained of, especially in the knees. In only one case did we see marked redness or swelling of the joints; in this one the wrists were involved.

Intercostal pain is very unusual and pain in the abdominal muscles is even less so; Guiteras and Cartaya likened the latter to the sensation produced by pressing a large iron on the abdomen. This description we have elicited a few times.

Skin eruption.—As previously stated, the face is usually deeply flushed and the eyes injected and watery at the onset of the disease. This appearance we have found very characteristic, and, in the circumstances under which we have worked, an almost pathognomonic sign. A similar appearance may be produced by so many beginning diseases that we would not give it any such weight where there was danger of confusing it with such diseases.

The redness may extend over the entire surface, but it is usually more marked on exposed parts, such as the face, neck, and hands. It is not a true eruption, but a general capillary dilatation and in appearance it resembles a mild sunburn, or the dilatation caused by a hot bath, rather than a scarlatinal rash. It may last for any length of time, from five or six hours to two or three days. It is not constantly observed and we have seen a few cases in which pallor was present instead.

We have in no case seen jaundice, neither did Guiteras and Cartaya, who, of course, kept it constantly in mind. These writers state that the skin is generally hyperæsthetic. We have not noticed such a condition and have had no complaints of it, so that we assume that it may vary in different epidemics.

The true rash undoubtedly varies greatly in the frequency of its occurrence, as well as in its duration and localization. We agree with Guiteras and Cartaya in regarding it as possibly present in all cases, though not noticed in all because it frequently is faint in appearance and of ephemeral duration. While we have not kept careful notes of all the patients we have examined, we think that we have seen the rash in about 75 per cent of our cases.

It most commonly appears about the fourth day, not infrequently with the terminal rise. At times we have seen it upon the third day, and at least twice in our experimental cases upon the second. As we received a majority of our cases, excepting those produced by inoculation, on the second, third, or fourth day, and as quite a number had the eruption when we first saw them, we could not determine accurately just when it did appear in some instances. We feel well satisfied, however, that the fourth or fifth day usually marks its first appearance.

The localization of the eruption varies. It occurs with greatest frequency, in our experience, on the trunk, either the anterior or posterior surface, or both, being involved. With this it may also appear on the wrists, ankles, neck, thighs, palms, or generalized over the entire body, the occurrence in the different locations being about in the order named.

The appearance of the rash also varies. The most common eruption more nearly resembles that of measles than any other well-known eruption, but it is not so dark in color, neither are the macules usually so coarse nor aggregated into such large patches. Another type resembles scarlatina, consisting of close set or coalescent, bright, red points, while between these two are intermediate types. Very rarely is the rash so vivid and plain as in scarlatina, measles or rubella. The measles-like eruption may be, at times, appreciable to the touch.

In some of the scarlatiniform eruptions the injection may be so intense as to produce capillary rupture and minute extravasations, which show on the bright-red background as small, purple dots. An eruption of this character is longer in fading than the others. These small extravasations are more commonly seen on the back and buttocks than elsewhere, possibly because of the greater heat and pressure to which these parts are subjected. In one patient (Case 10) these small extravasations apparently suppurated; at all events, an abundant crop of miliary pustules, 1 to 3 millimeters in diameter, appeared over the buttocks, where the extravasations were abundant. The pustules were not painful and gave rise to no symptoms.

Occasionally, the eruption leaves small areas of skin, from 1 to 2 centimeters in diameter, uninvolved, which then present somewhat the appearance of wheals on a blushing surface. We have not seen an urticarial eruption.

The duration of the rash usually varies with its intensity, the well-marked eruptions lasting longer than the others, and, as stated, the scarlatiniform rash with extravasations the longest. In one such case (Case 2) the eruption lasted eight days. We have seen no other in which it lasted so long, though we have observed others in which it was visible for a week. In most cases it lasts about two days; that is, it appears on the fourth day, or the fifth and disappears by the time the temperature falls, on the sixth day. In many cases it lasts only one day, or possibly less, being well marked one morning and absent the next. In about one-fourth of our cases it was never seen at all, and possibly did not occur.

The disappearance of the rash in a minority of cases is followed by a fine desquamation which will not be noticed unless watched for closely.

In a very small minority the desquamation is easily observed as fine, bran-like, but abundant, scales. In one patient whom we saw but did not have under our care, the skin of the hands, arms and feet came off in large strips, many of them an inch square.

Alimentary system.—The tongue in nearly all cases presents a characteristic appearance. At first it is covered by a light, creamlike coat which rapidly thickens and darkens in the middle, disappearing from the edges; during the rest of the attack the tongue usually presents a heavy, yellowish central coat, with a red tip and edges. It remains moist throughout and shows no tendency to fissure. The breath is heavy and at times foul, especially in cases showing constipation.

The appetite is practically always impaired or absent for the first few days. By the third or fourth day most of our patients were very hungry and asked for full diet, which all but two or three of them relished.

Nausea and vomiting occurred in a few cases, as did diarrhœa. This last was profuse and watery, but we never saw either mucus or blood in the stools, though both are said to occur at times. The vomiting and diarrhœa which we observed always occurred at the onset of the disease, and not as manifestations of the crisis.

As a rule, slight constipation is present, necessitating the administration of laxatives. As our patients were all soldiers leading active lives and taking much exercise, it is not improbable that the inaction of hospital confinement had as much influence as the disease in producing the constipation.

Nervous symptoms.—The most constant of these, the pains, have already been discussed. In three cases we have had delirium, that in one was very mild, in another slightly more marked, and in the third attended with marked hysterical symptoms and hallucinations. In all three cases the delirium was observed only at night, and in two it occurred as the patient was falling to sleep and may have been merely the manifestation of troubled dreams. The other case, Case 10, was as severe a one as we saw, but even in it the symptoms pointed rather to hysteria than to meningitis, and we afterwards learned that the patient had for several years, been subject to nervous attacks, beginning when he was a small boy and continuing until about two years ago. He also had an attack, similar to the one he showed during his fever, a short time after his return to duty. In this attack he got out of bed, ran about the room, shouted, wept and talked to his mother, who was of course not present. He was quieted, and the next day was impressed with the folly of his conduct and the necessity for maintaining self-control. Since that time, now nearly three months, he has had no trouble, and has performed his full duty.

We have not seen the "meningeal type" of the disease as it has been described by others, nor have we heard of any such cases occurring during this epidemic. We have not seen peripheral neuritis, unless some of the pains were due to such lesions, in which case the neuritis did not outlast the other symptoms and must have been trifling.

Insomnia was frequently observed while the fever was high and the pain severe. It was so evidently dependent on these causes and disappeared so soon that it did not require treatment.

Disturbances of the circulatory apparatus should probably be considered here. A considerable minority of patients complained of pain or dis-comfort in the præcordium or of a sense of suffocation, and one man had an attack of syncope while eating. In none of these cases were we able to discover any sign of heart, lung, or pericardial lesion. Not even the cardiac rhythm was disturbed during any of our examinations.

However, the frequency of complaint, and the fact that one man actually fainted, show that the circulation is often disturbed in this disease, and this disturbance prompts us to join with other writers in pointing out the necessity for guarding against accidents from such a cause.

Hæmorrhages.—Several writers speak of the tendency to hæmorrhages, and Guiteras and Cartaya, whose cases were many of them sent to the hospital as yellow fever suspects, noted it in almost a fifth of the cases they studied. We did not observe hæmorrhages in any instance, if we except the small capillary ruptures described as an occasional feature of the eruption.

The possibility of the occurrence of hæmorrhages from other parts should be kept in mind, especially in yellow fever countries, for though Guiteras and Cartaya observed none from the stomach or bowels, other writers say they may occur.

Lymphatic glands.—It is stated by some observers that the lymphatic glands show an enlargement during this disease. This observation we can not confirm, as we saw no lymphatic enlargements except in cases which had shown them prior to the onset of dengue, or who developed inguinal adenitis from coexisting venereal disease.

Blood.—The results of the examination of the blood have already been considered.

Urine.—Guiteras and Cartaya state that albumen may often be found in the urine if very delicate tests are used, they detecting it in 41 per cent of their cases. We have never seen any symptoms referable to the urinary system except in a few men who had a coexisting gonorrhœa, and we therefore did not have the urine examined in many instances. We had eight urines examined, and they were all normal but one. However, our experience indicates that in some epidemics the condition of the urine gives more valuable information for a differential diagnosis between dengue and yellow fever than Guiteras and Cartaya thought.

Convalescence.—Many writers state that convalescence is often prolonged and tedious, and apt to be protracted by such complications as boils, joint affections, muscular pains, or weakness in the knees. In all of our cases convalescence has been prompt, practically all patients expressing a desire to return to duty as soon as the temperature fell.

Mortality.—All observers agree in saying that the mortality in dengue is so low as to be almost nothing. During the Australian epidemic 94 deaths occurred from the disease in Brisbane. The Robertson committee estimated that this represented about one death in 1,000 cases;

the mortality was relatively greater in females than in males, and was highest at the extremes of life; patients under 5 years of age contributing 37.6 per cent of all deaths, those over 60 years 35.5 per cent.

We have seen no deaths, and have heard of none during the epidemic we have studied. The disease does its harm from a military standpoint by disabling such large numbers of men at one time. When 600 men out of 1,000 or 1,200 are disabled for a period of at least a week each, the work of the command must, of course, suffer.

V. DIAGNOSIS.

As indicated in the consideration of the symptoms, the diagnosis of dengue will often not be made except in the presence of an epidmic, in which case the tendency would probably be to call any painful affection of sudden onset "dengue."

The fairly characteristic temperature chart, the sudden onset, severe pain, flushed face, the coated tongue, the eruption, and the leucopenia and lymphocytosis, unite to make the ordinary case easy of diagnosis, especially in the presence of an epidemic.

Care is required, under various conditions, in differentiating dengue from yellow fever, malaria, influenza, scarlet fever, measles, syphilis, tonsilitis, rheumatism, smallpox, and meningitis.

Yellow fever.—The differential diagnosis between yellow fever and dengue is probably the most important we have to consider, as the two diseases occur side by side in America, and mistaken diagnosis might lead to the gravest consequences, as a supposed dengue case is not apt to be so carefully guarded from mosquitoes as is a known one of yellow fever.

Guiteras and Cartaya, experienced in both diseases, say that the most valuable differential signs are the slower pulse, the jaundice and the haematemesis in yellow fever. None of these are apt to occur in dengue. Add to this the greater liability to albuminuria in yellow fever, the character of the prevailing epidemic, the mortality, the absence of the eruption and probably the blood examination, which in yellow fever does not show the characteristic leucopenia and lymphocytosis of dengue, and in the great majority of cases the diagnosis will be clear. Nevertheless, it would be the part of wisdom in all doubtful cases to act as though the disease were yellow fever.

Malaria.—The history and the microscope will usually make an early differentiation possible. In case they do not do so, quinine will do little harm.

Influenza.—The geographical and seasonal distribution of the two diseases do not correspond. Dengue occurs only with the mosquitoes, influenza where mosquitoes are absent and oftenest in cold weather. Influenza is usually accompanied by catarrhal symptoms, dengue rarely so, and then only accidentally. Dengue usually shows an eruption of a scarlatinal or rubeoloid type, influenza does not. Leucopenia and lymphocytosis point more strongly to dengue.

Scarlatina.—The occurrence or nature of the epidemic, the seasonal occurrence, the almost entire absence of sore throat and cervical glandular swelling, the age of the patient, the less marked toxic symptoms, the temperature chart, the leucocyte count and the usually slight desquamation, will in nearly all instances set dengue apart from scarlatina.

Measles.—The more sudden onset of dengue, the greater pain, the absence of coryza, the appearance of the temperature chart, the epidemic and its season, usually makes this differentiation easy.

Syphilis.—Confusion with syphilis will occur but rarely, and only in individual cases. In such cases the history, the chart, the usually less violent onset of symptoms, the examination for chancre, mucous patches, etc., will practically always enable one to make a diagnosis.

Tonsilitis.—The onset of acute, follicular tonsilitis is at times, in its suddenness, its painfulness and fever, much like that of dengue. The examination of the throat is usually sufficient for the making of a correct diagnosis.

Rheumatic fever.—Acute articular rheumatism is at times, but unusually, simulated by dengue. In the latter disease the joint involvement, when present, is less marked and more ephemeral, while the other dengue symptoms, especially the eruption, make the diagnosis clear.

Smallpox.—The sudden onset, the flushed face, the violent pain in the head and back, the high temperature, make the early stages of smallpox resemble those of dengue, and it is probable that smallpox developing during dengue epidemics will often be mistaken for it. The history of exposure to smallpox, the absence or great age of vaccination scars, would point to that disease, while the evolution of the pocks would soon put the case beyond the realm of doubt.

Meningitis.—Some of the cases of the "meningeal" type of dengue, as described by other writers, can probably only be differentiated from epidemic cerebro-spinal meningitis by the presence of the dengue epidemic and the result of the bacteriological examinations of the fluid obtained by spinal puncture.

VI. TREATMENT.

Prophylaxis.—We believe that our observations and the total failure of all our attempts to transmit the disease by fomites or contact indicate the character of the prophylactic measures. Protection from mosquitoes is probably all that is necessary, but there is the posibility that other transmission of infected blood may rarely occur, as for example through other insects, infected hypodermic needles, and in a few other possible but improbable ways. We believe that in the case of the military service the screening of barracks would prevent such epidemics as the one which occurred at Fort William McKinley, and it would appear that economy would be subserved by the screening of all barracks and quarters in countries where malaria and dengue are prevalent.

Medicinal.—Our cases did not receive treatment except in a few instances and for special symptoms, as we wished to obtain a picture of the unaltered disease. To judge from our observations on these untreated cases we think that other than symptomatic treatment, to promote the patient's comfort, is not called for.

Cold bathing, sponging, and the use of ice-caps are advisable to keep the temperature within bounds; for the pain and nervous symptoms, opium and bromides would probably be safer routine measures than the use of the coal-tar products; because, first, in this disease they are more effective, and, second, they would be less apt to harm an already disturbed heart.

VII. CONCLUSION.

In concluding our report we desire to express our appreciation of the encouragement and aid rendered us by Maj. Gen. Leonard Wood, commanding the Philippines Division, without which it would have been impossible for us to have made these researches. We also desire to thank Dr. R. P. Strong, the Director of the Biological Laboratory of the Bureau of Science, for the use of apparatus, and Mr. Charles S. Banks, Entomologist of the Biological Laboratory, Bureau of Science, who rendered us assistance in the identification of mosquitoes.

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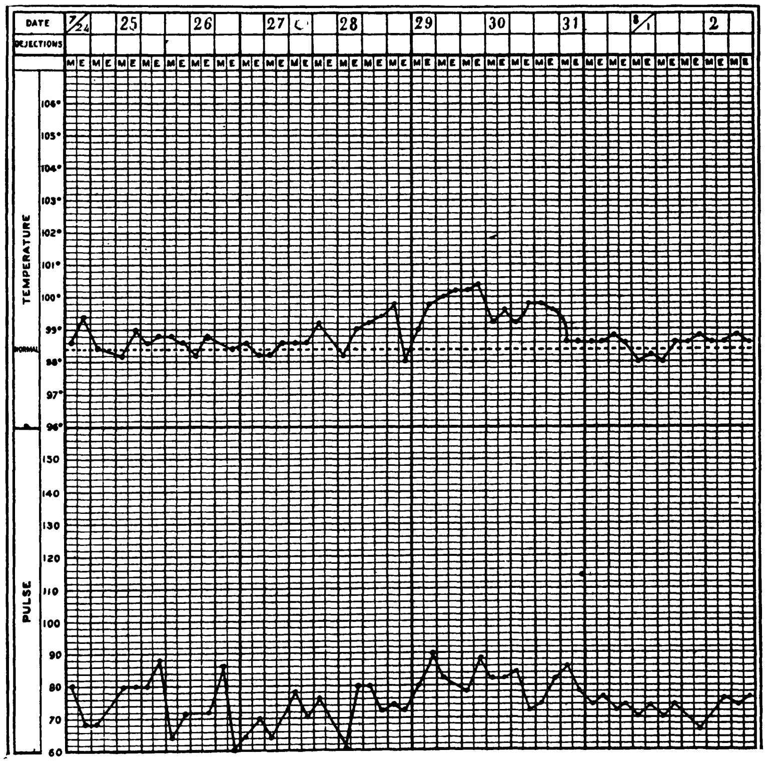

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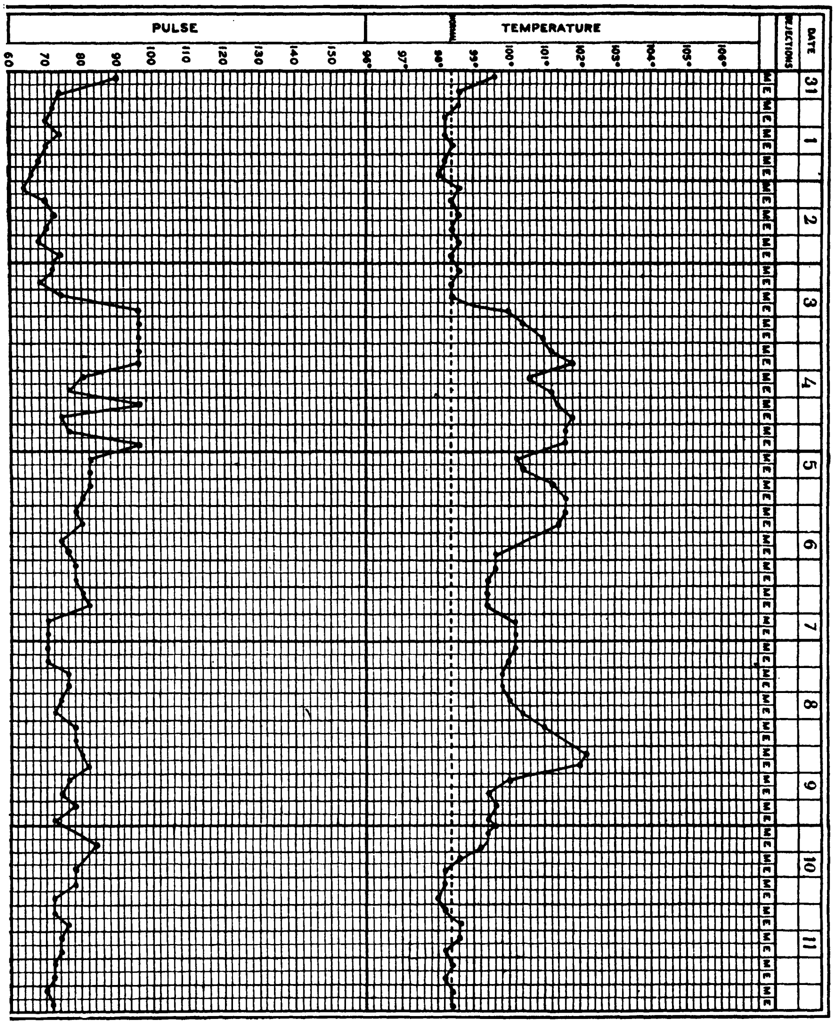
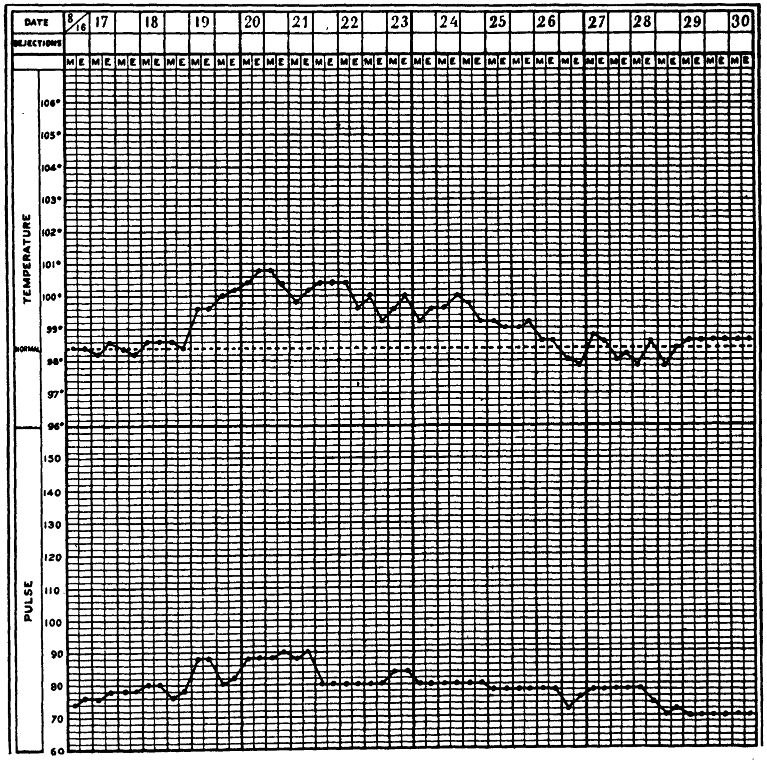


Case 2, Chart 2.



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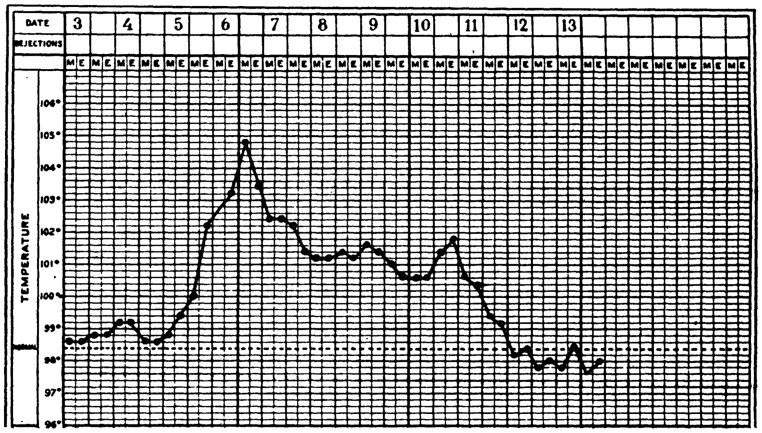

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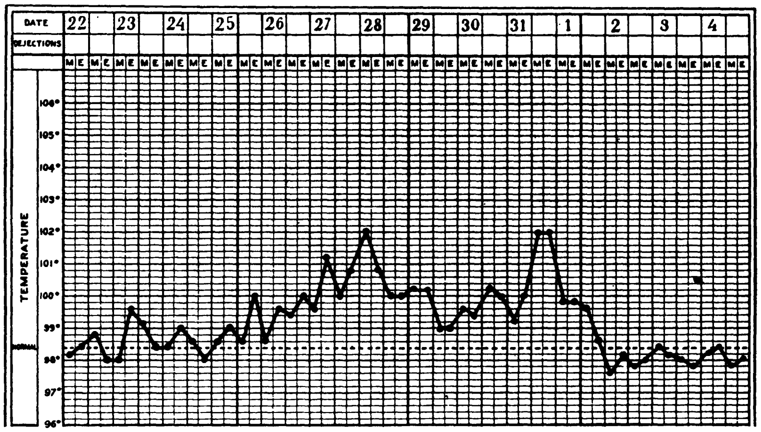

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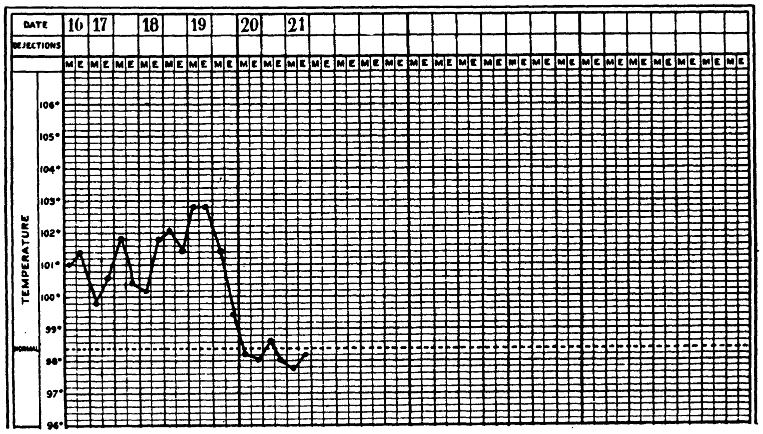

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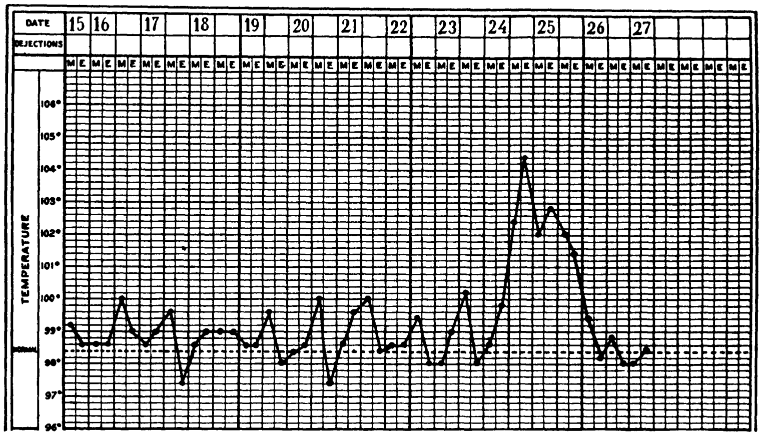

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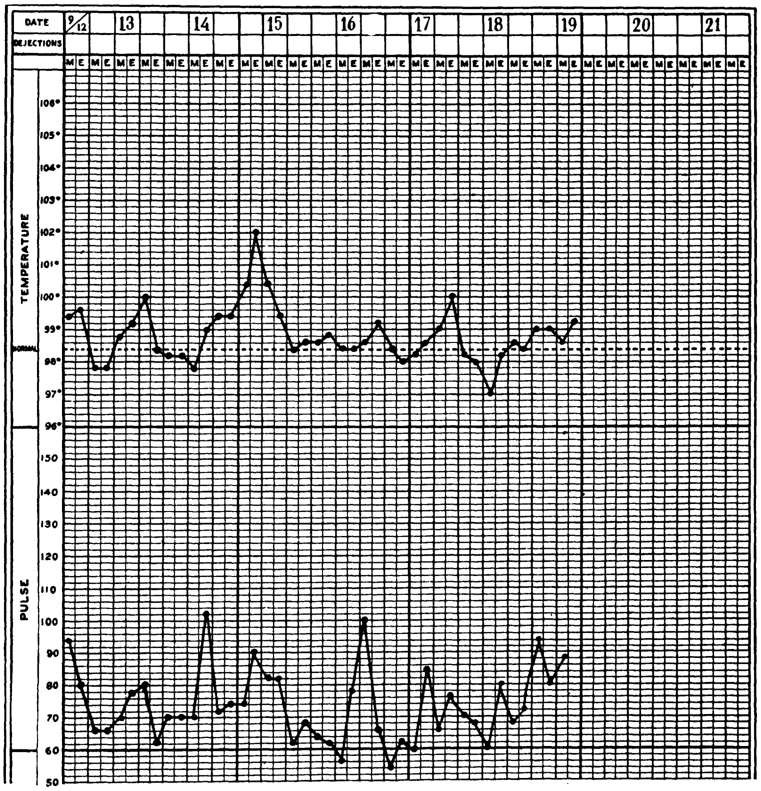

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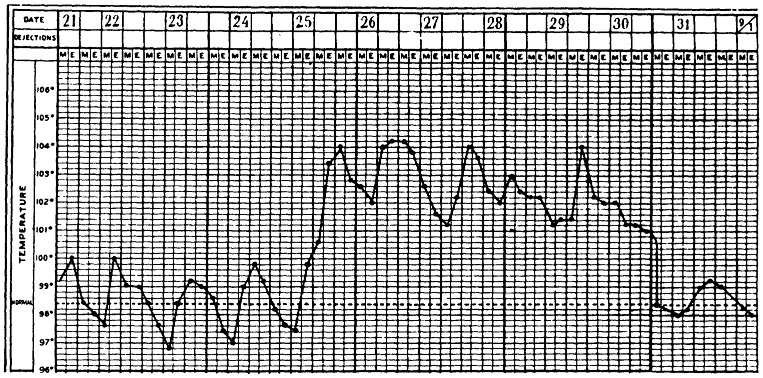

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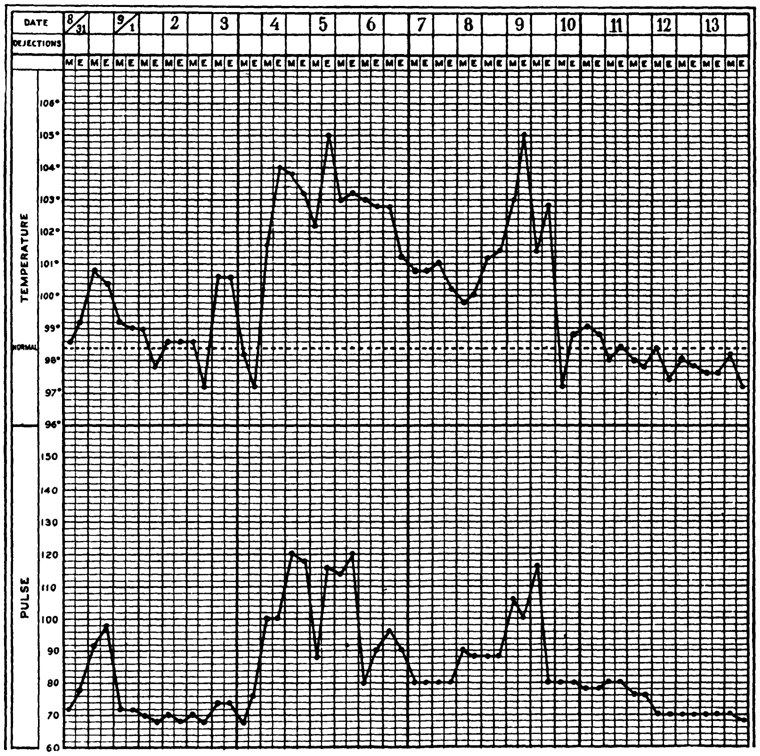
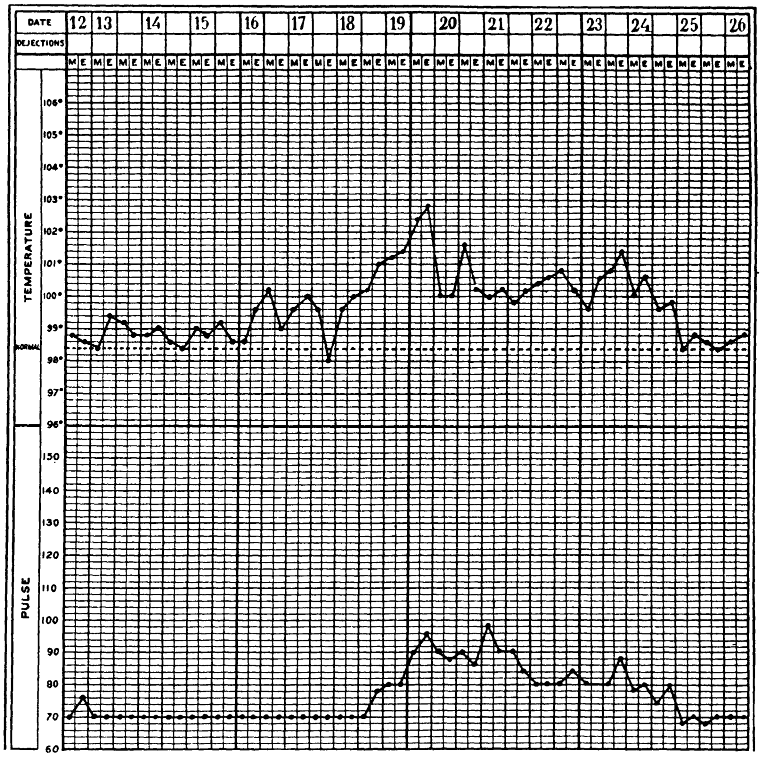
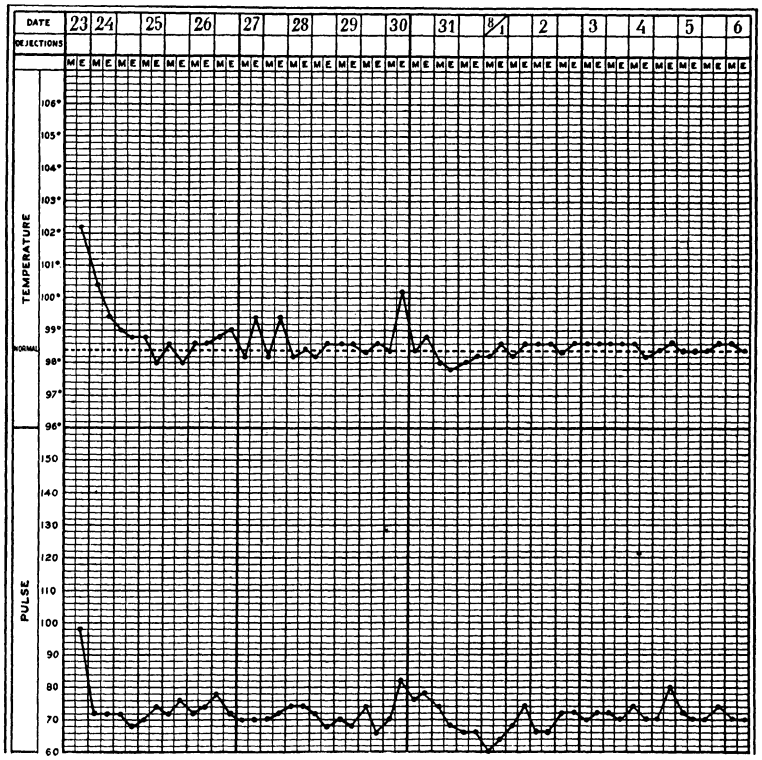


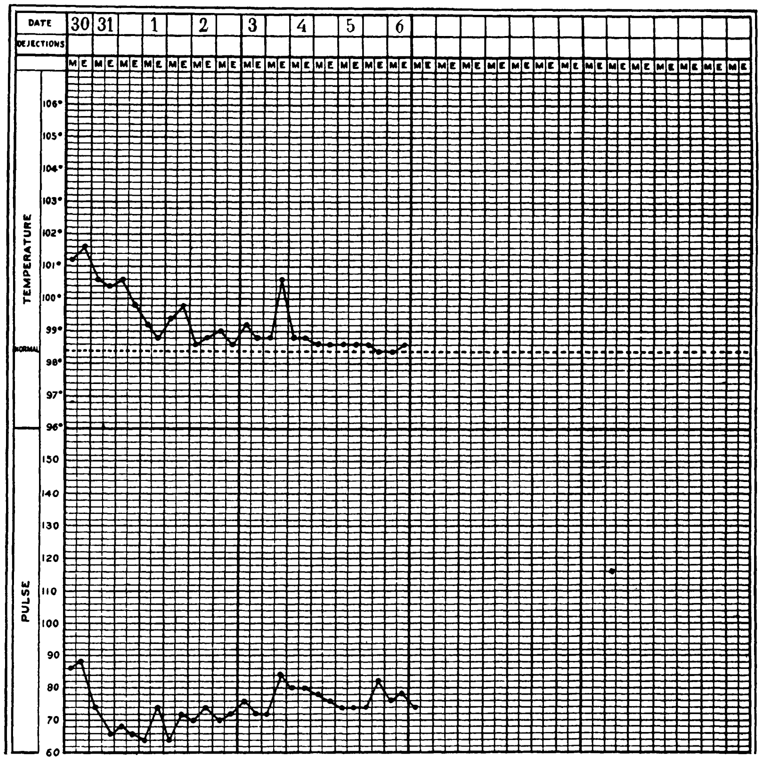
Case 10, Chart 10.



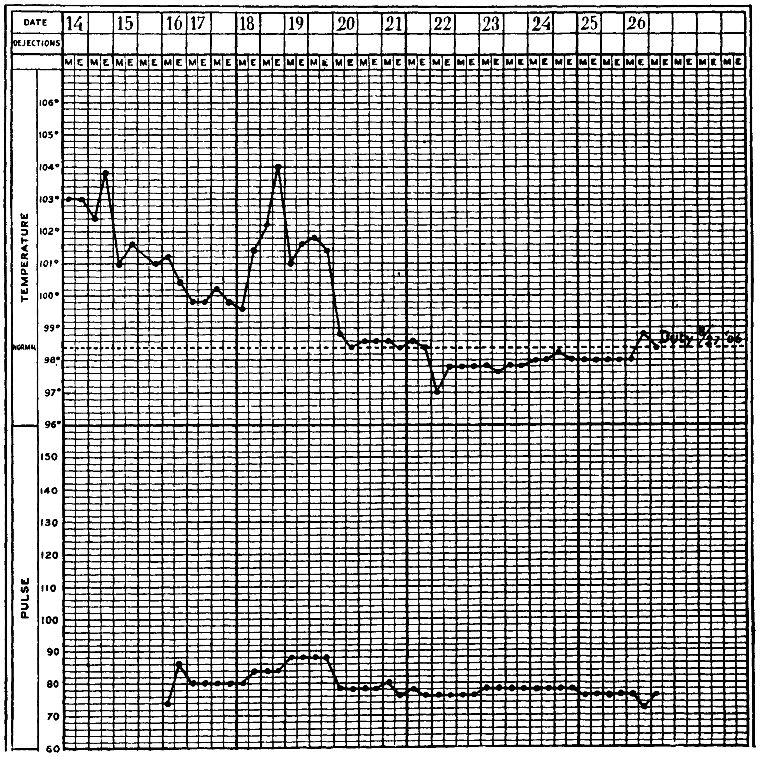
Case 11, Chart 11.



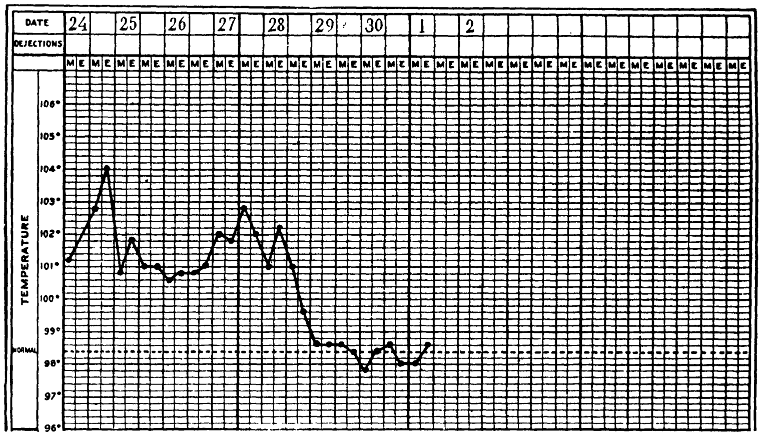
Case 20, Chart A.



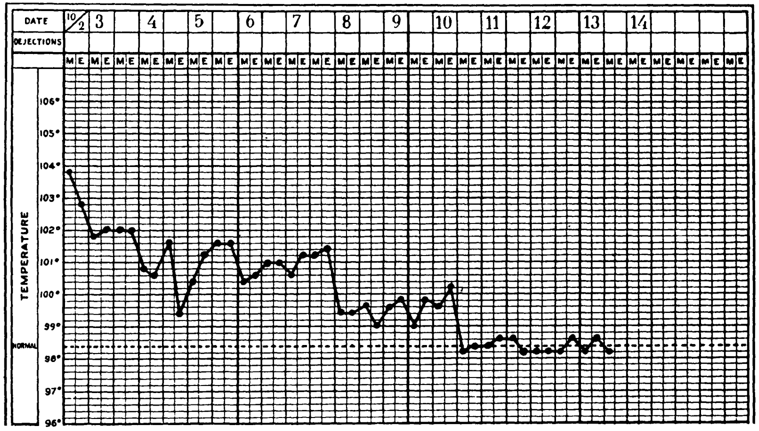
CASE 30, CHART B.



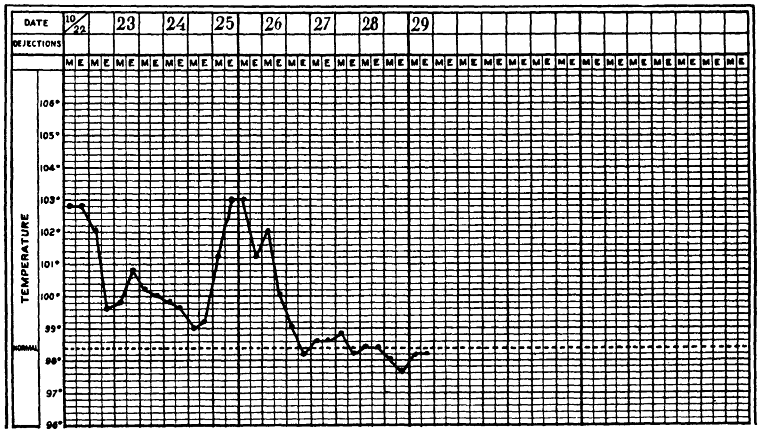
CASE 36, CHART C.



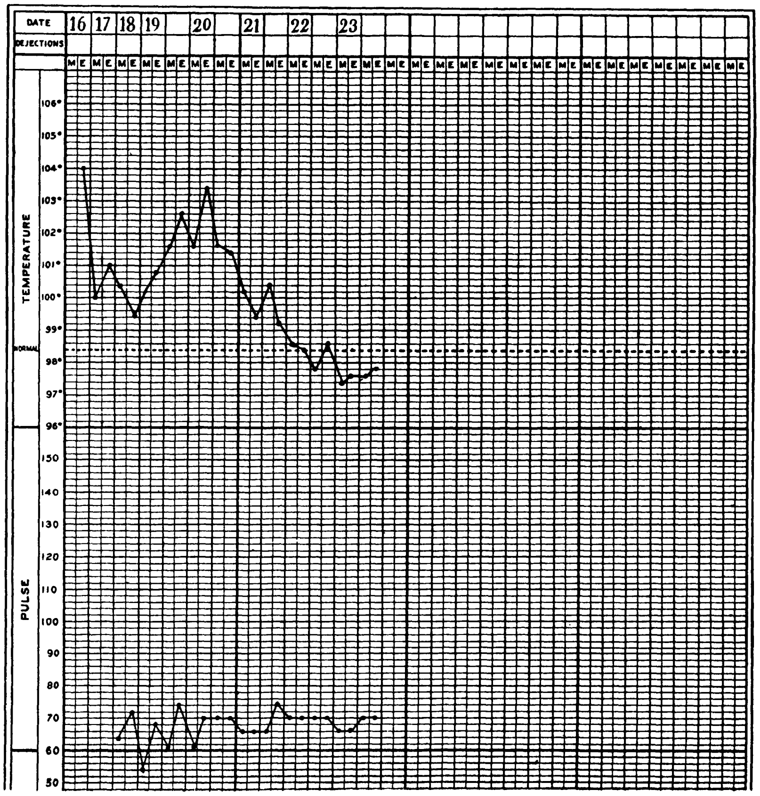
Case 38, Chart D.



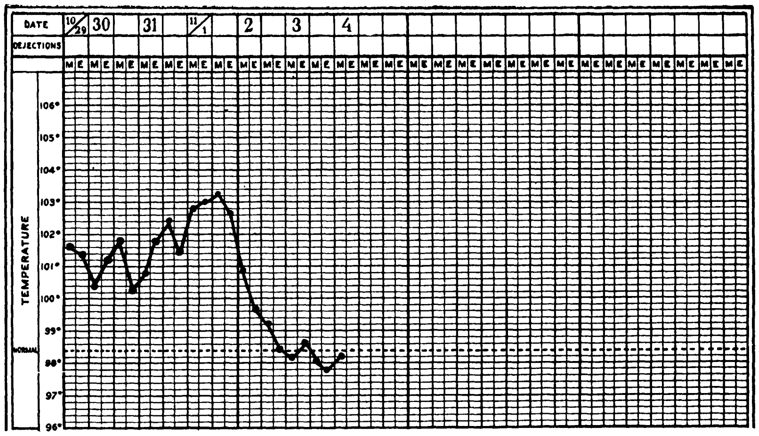
Case 44, Chart E.



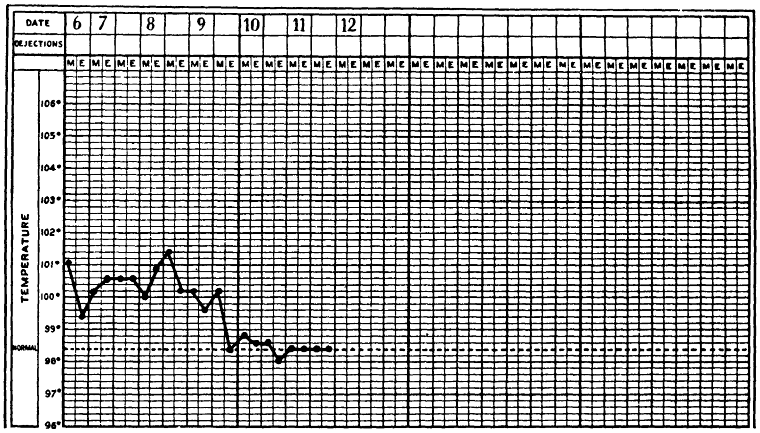
Case 60, Chart F.



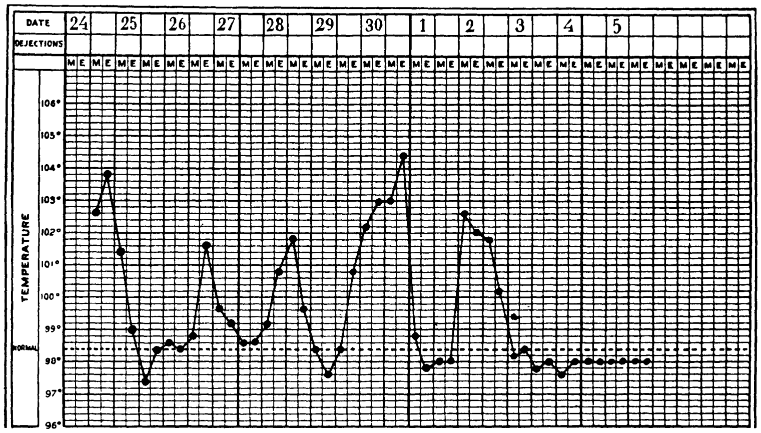
CASE 41, CHART G.



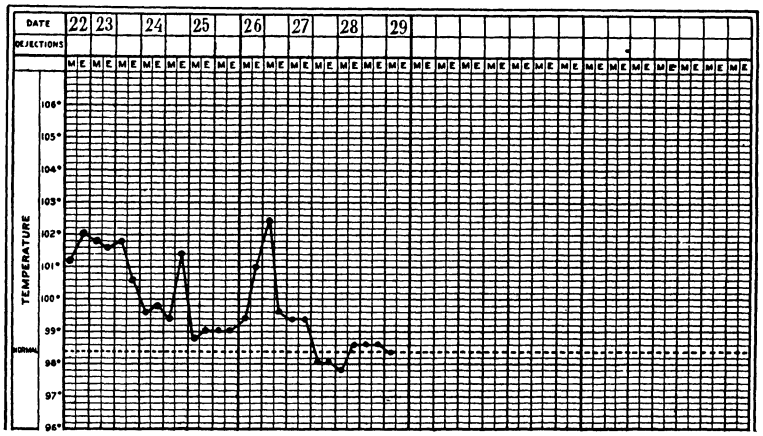
CASE 65, CHART J.



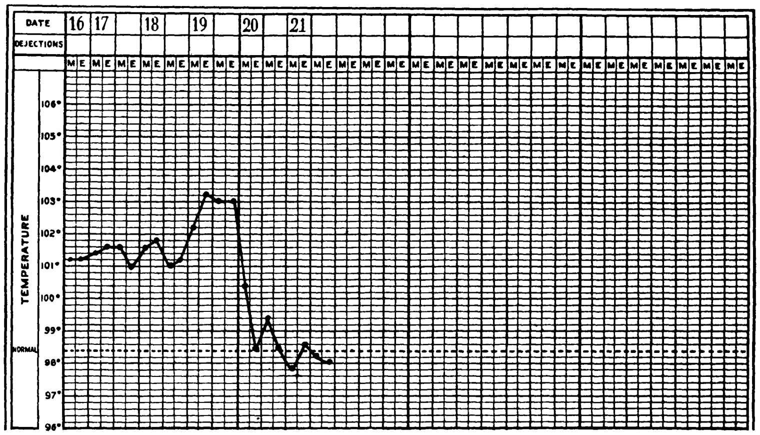
Case 70, Chart K.



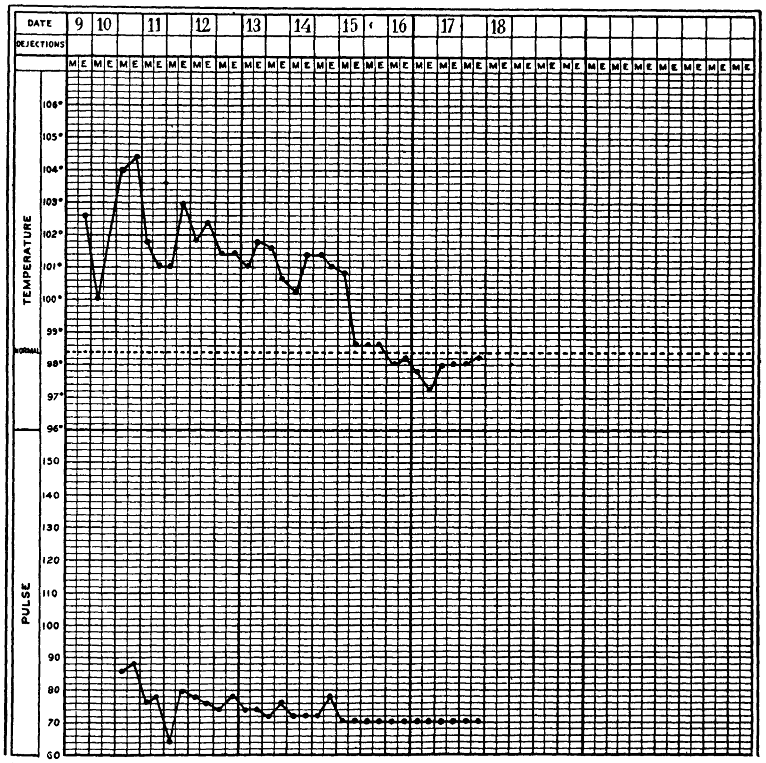
Case 80, Chart L.



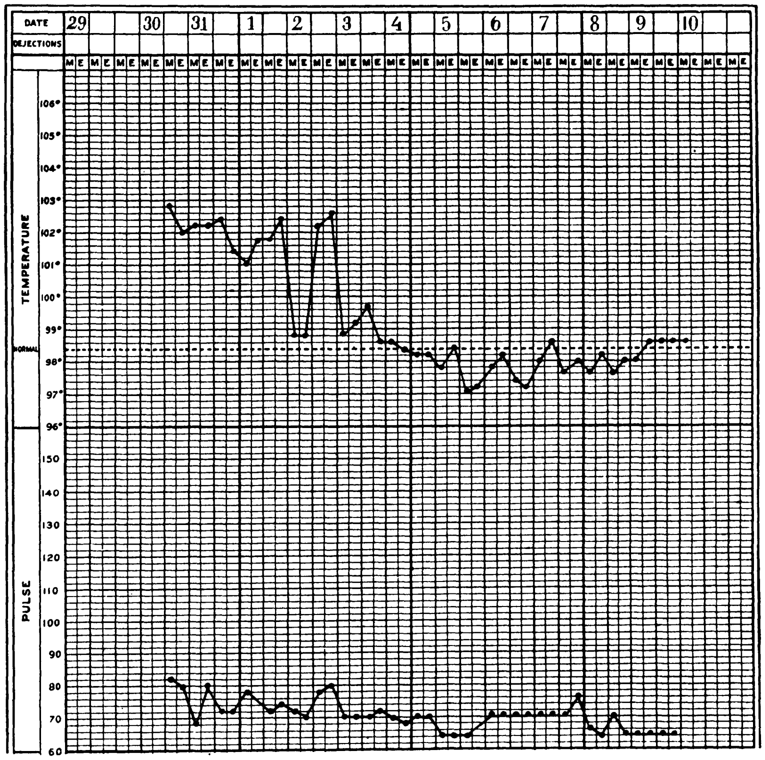
Case 81, Chart M.



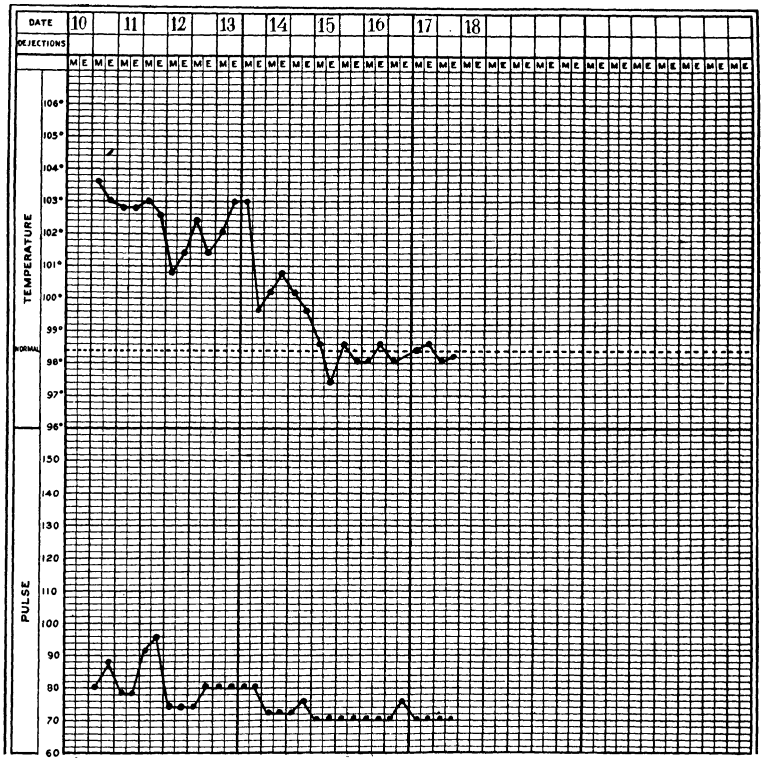
CASE 82, CHART N.



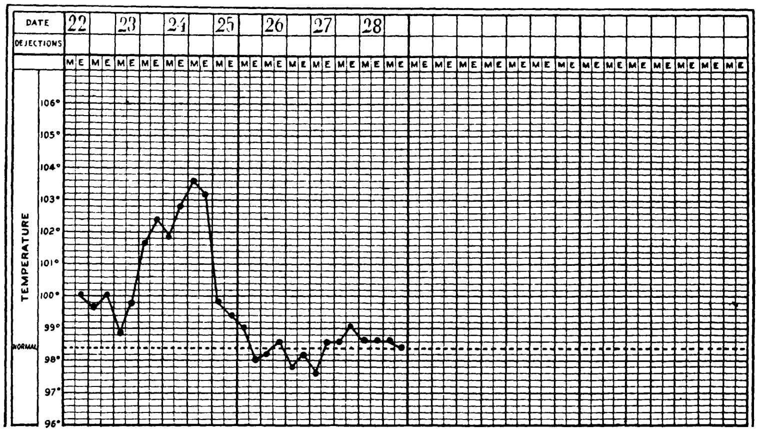
CASE 83, CHART O.



CASE 87, CHART H.



CASE 88, CHART R.



CASE 95, CHART S.

MAP.

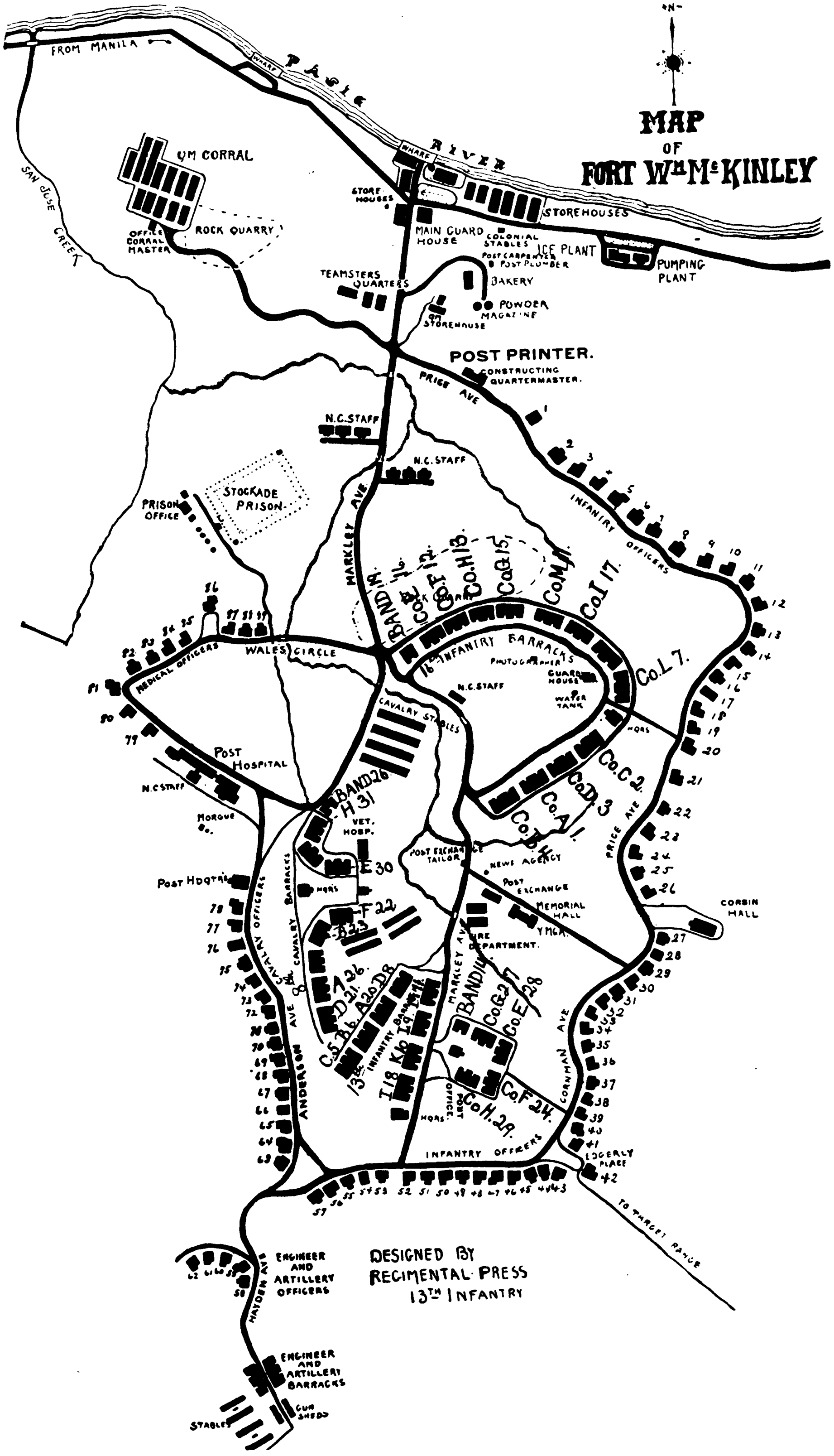
Sketch map of Fort William McKinley, Rizal Province, P. I. 151

EXPLANATION OF MAP.

The map is intended to illustrate the spread of the epidemic of dengue through the post of Fort William McKinley, Rizal Province, P. I.

The letters placed opposite the barracks indicate the company occupying them, and the figures accompanying the letters indicate the order in which the barracks were infected. It will be observed that barracks widely separated became infected before others in apposition, and that the last barracks to become infected were those of the Eighth Cavalry.

The red line indicates the course of a small stream of water which is an ideal breeding place for mosquitoes.


N
MAP OF FORT Wm McKINLEY
PASIG RIVER
FROM MANILA
WHARF
WHARF
QM CORRAL
SAN JOSE CREEK
OFFICE CORRAL MASTER
ROCK QUARRY
STORE HOUSES
STOREHOUSES
MAIN GUARD HOUSE
COLONIAL STABLES
POST CARPENTER
POST PLUMBER
ICE PLANT
PUMPING PLANT
TEAMSTERS QUARTERS
BAKERY
POWDER MAGAZINE
QM STOREHOUSE
POST PRINTER.
CONSTRUCTING QUARTERMASTER.
PRICE AVE
N.C. STAFF
N.C. STAFF
STOCKADE PRISON
PRISON OFFICE
MARKLEY AVE
INFANTRY OFFICERS
BAND 16
Co.E 12
Co.F 10
Co.H 13
Co.G 15
Co.M 11
Co.I 17
Co.L 7
ROCK QUARRY
16th INFANTRY BARRACKS
PHOTOGRAPHER
GUARD HOUSE
WATER TANK
N.C. STAFF
HQRS
Co.C 2
Co.D 3
Co.A 1
Co.B 4
WALES CIRCLE
MEDICAL OFFICERS
POST HOSPITAL
N.C. STAFF
MORGUE
CAVALRY STABLES
BAND 26
H 31
VET. HOSP.
E 30
HQRS
F 22
B 23
A 26
D 21
C 5
B 6
A 20
D 8
8th CAVALRY BARRACKS
CAVALRY OFFICERS
ANDERSON AVE
POST HDQTRS
POST EXCHANGE TAILOR
NEWS AGENCY
POST EXCHANGE
MEMORIAL HALL
Y.M.C.A.
FIRE DEPARTMENT.
CORBIN HALL
MARKLEY AVE
BAND 14
Co.G 27
Co.E 28
13th INFANTRY BARRACKS
I 18
K 10
L 9
M 11
POST OFFICE
HQRS
Co.H 29
Co.F 24
CORNMAN AVE
INFANTRY OFFICERS
EDGERLY PLACE
TO TARGET RANGE
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89
HAYDEN AVE
ENGINEER AND ARTILLERY OFFICERS
ENGINEER AND ARTILLERY BARRACKS
GUN SHEDS
STABLES
DESIGNED BY REGIMENTAL PRESS 13th INFANTRY

REVIEWS.

A Text-Book of Pathology. By Alfred Stengel, M. D. With 399 illustrations in the text, many of them in colors, and 7 full-page chromolithographic plates. Fifth edition, thoroughly revised. Cloth. Pp. 979. Price, $5.00 net; half morocco, $6.00 net. Philadelphia and London: W. B. Saunders Company, 1906.

It is obvious that a work upon pathology which has reached a fifth edition in a period of eight years must be possessed of more than ordinary merit, and have answered the needs of those who have consulted it. Written from the standpoint of the clinical pathologist, the subjects, in the work under review are presented in such a manner as to be of the greatest service to the practitioner and student of medicine, and taken as a whole, the work is one of the most valuable treatises upon pathology contributed by an American author. This edition is well up to date and much recent work has been incorporated or summarized; however, we greatly miss references to the literature of the various subjects discussed, especially in that portion of the work devoted to the animal and vegetable parasites, where a few well-chosen references would have proven of great value to the student.

The first six chapters of the work are devoted to general pathology, and are most excellent, especially the chapter upon progressive tissue changes, in which is given a beautifully illustrated and concise description of the histo-pathology of tumors.

Chapters 7 and 8 are devoted to the bacteria and the diseases due to them and to the animal parasites. In the reviewer's opinion the inclusion in a text-book of pathology of anything like an adequate consideration of the vegetable and animal parasites infecting man is impossible and might better be altogether omitted, but, unfortunately, it has become a custom to attempt to do so, and the chapters referred to are very satisfactory and the subjects are presented in an attractive manner; the author has accepted the classification of amœbæ as defined by Schaudinn, and includes the *Spirochætæ* under the bacteria. As regards the position of the *Spirochætæ,* it would have been better to have regarded their biological position as uncertain, especially as the recent work of Breinl appears to have disproven that of the adherents of the bacterial theory and again swung the pendulum toward the Protozoa. The illustrations in this section of the work are as a rule good, but many of them are reproductions of old and imperfect woodcuts; the photomicrograph given of *Treponema pallidum* could much better have been used to represent *"refringens"* for it is not at all typical of *"pallidum."*

The remainder of the work is devoted to special pathology, is well illustrated, and presents the subject in an interesting and instructive manner; owing, undoubtedly, to lack of space, many important subjects are merely mentioned, or the descriptions are so brief as to be of little practical value. The pathology of the diseases of the Tropics has suffered more in this way than any other branch of medicine, as an instance of which may be mentioned the description of the pathology of dysentery; no distinction is made between the pathology of specific or bacillary dysentery and the amœbic variety, and the Shiga bacillus is not even mentioned by name, but referred to as "a bacillus resembling the typhoid bacillus." It is unfortunate that so excellent a work as the one before us should be marred by so unsatisfactory a description of the pathology of this important group of infections.

The book is carefully indexed and is well printed and bound.

C. F. C.

The Elements of the Science of Nutrition. By Graham Lusk, Ph. D., M. A., F. R. S. (Edin.) Illustrated. Cloth. Pp. 326. Price $2.50 net. Philadelphia and London: W. B. Saunders Company, 1906.

Professor Lusk has given a very clear and comprehensive review of the known facts about the very complex subject of nutrition, including the results of his own careful work. The book deals largely with the scientific side of the subject and contains little of the practical details usually prominent in books upon this subject. It is to be hoped that the study of such books as this one together with the works of Pawlaw, Atwater, Chittenden and others who are doing so much toward elucidating the problems of nutrition will lead to the more general practice of this subject upon a scientific basis.

W. E. M.

THE PHILIPPINE
JOURNAL OF SCIENCE
B. MEDICAL SCIENCES

VOL. II JUNE, 1907 No. 3

STUDIES IN PLAGUE IMMUNITY.[1]

By RICHARD P. STRONG.
(From the Biological Laboratory, Bureau of Science.)

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[1] Read by abstract at the Fourth Annual Meeting of the Philippine Islands Medical Association March 2, 1907.

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I. INTRODUCTION.

The experimental work which constitutes the basis of this report was undertaken with the object of throwing further light upon certain practical problems in relation to pest immunity, and particularly with the idea of determining the most efficacious method of protective inoculation against plague. The subject of immunization against pest is not only of general scientific interest, but at least to several tropical and subtropical countries is of great practical importance. One need only recall the mortality in India of nearly a million deaths from this disease during the past year (1905) to be impressed with the importance of the problem, and while it is true that at present no epidemic of plague exists in the Philippine Islands, yet sporadic cases occur from time to time and it must be recalled that only a few years ago (1903) it was thought by the Board of Health advisable and necessary to perform among the Chinese of the city of Manila general inoculations against this disease.

It is true that the subject of protective inoculation against plague has received considerable attention during the past few years and that prophylactics have been recommended by several authors, but, while it is admitted that by their use a certain degree of pest immunity can be produced and demonstrated in a number of the more insusceptible animals and, occasionally, even in those very susceptible to this infection, nevertheless, it has sometimes seemed questionable whether we were able, by the inocultion of these prophylactics, to obtain in man an immunity of such a degree as to be protective against the natural and usual methods of infection by the malady. Thus, Kolle called attention to the fact that since, by the use of even very large doses of the killed pest organisms only exceptionaly were we able to immunize against pest infection the animal which is most susceptible to plague, namely, the guinea pig, it seemed very doubtful if favorable results could be obtained in man, where relatively much smaller doses of the killed cultures were injected. Yet, from 1898, when protective inoculation was first introduced, up to the time of my first report on vaccination against plague in November, 1905, no other methods except those in which the killed cultures were employed had been used in the very extensive human inoculations performed against this disease in India and in other countries.

In the latter part of the year 1903, when the Board of Health of Manila was practicing among the Chinese in this city protective inoculation against plague by the injection of the killed cultures of the pest bacillus, the method at that time carried on in Japan and consisting of the injection of 1 oese of a 24-hour slant agar culture of *Bacillus pestis* suspended and killed in 1 cubic centimeter of 0.085 saline solution, I decided to investigate whether any immune substances became developed in the serum of the inoculated individuals. It did not seem possible to me that any appreciable degree of immunity could be acquired from these inoculations, owing to the small size of the dose and the very mild local and general reaction which resulted from the injections. I therefore studied the agglutinative and bactericidal reactions of the blood serum of 12 cases, 6 of whom had been inoculated two weeks and 6 three weeks previously with 1 oese of the killed pest culture. The agglutinative reactions were performed by the macroscopic method and the bactericidal reactions according to the one suggested by Neisser and Wechsberg. No traces of agglutinins or of bacteriolysins could be demonstrated in the sera of any of the individuals.[2] Obviously, these experiments in themselves were not considered to be conclusive evidence of the fact that no immunity was conferred upon the inoculated, since it was already recognized at this time that these antibodies were frequently and indeed, usually, not encountered even in the blood sera of individuals who had recovered from an attack of plague and were immune to this disease.[3] Therefore, experiments in animals were resorted to in order that more information on this subject might be obtained. Ten guinea pigs were inoculated subcutaneously, each with the same dose that was being employed in the general human inoculations in this city. After two weeks the immunity of these animals was tested in the following manner. One oese of a virulent pest organism was suspended in 1 cubic centimeter of 0.085 saline solution and 5 oesen of this suspension rubbed over a freshly shaved area on the abdomen of the guinea pig. All of the animals succumbed to acute pest infection, demonstrating conclusively that an immunity of appreciable degree had not been produced. A short time after, the important paper of Kolle and Otto[4] was published in which the unfavorable results from the immunization of guinea pigs with large doses of killed agar cultures of the pest bacillus or with Haffkine's prophylactic were reported. It therefore seemed to me, at that time, more advisable to experiment further with other methods of immunization against plague, before insisting upon the use of larger amounts of more virulent killed pest cultures in the human inoculations being pursued in this city. Further results obtained by Kolle and his associates in Berlin, and the animal experiments made here, having demonstrated that in very susceptible animals at least, satisfactory immunization could only exceptionally be produced by the methods of human inoculation then in vogue, this laboratory recommended to the Commissioner of Health in Manila the suspension of the human inoculations with killed cultures of the pest bacillus, and has not since recommended to our Government any method of protective inoculation against plague as an efficient procedure against the extension of this disease. However, further experimentation, which I have carried on as continuously as was practicable for the past year and a half, has demonstrated that a satisfactory immunity can be obtained in the guinea pig, *an animal even more susceptible to plague infection than is man,* by the inoculation of living cultures of plague bacilli of such attenuation that they are no longer dangerous when injected into human beings. Therefore, this laboratory believes that by the method of *vaccination* [5] against plague with suitable cultures, we have probably an efficient measure for the control of this infectious disease, and we recommend its adoption for this purpose.

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[2] These experiments were undertaken at this time because the statement had previously been made that agglutinins, at least in some cases, had been demonstrated in the blood serum of human beings who had been inoculated against plague a short time before with Haffkine's prophylactic, a conclusion which I have not been able in any manner to confirm.

[3] Experiments demonstrating the fact that animals immune to pest infection may still show no traces of agglutinins in their blood, together with those demonstrating the absence of a true bacteriolytic action of plague immune serum, will be presented later in this paper.

[4] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1903), **45**, 507.

At present there is not sufficient plague in this city to warrant the employment of general vaccination against the malady, but it is hoped that the method may be given a thorough and careful trial in certain districts of India where plague is always present in sufficient amounts to justify its use. A preliminary report on the subject of vaccination against plague was presented to the Manila Medical Society in November, 1905, and published in an earlier number of this JOURNAL.[6] In the present article further evidence of the entire safety of vaccination in man against plague with suitable cultures will be presented, together with the experimental work which demonstrates conclusively the efficacy of this procedure and its superiority to other methods of protective inoculation, including those in which both natural and artificial plague aggressin, so recently described, are employed.

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[5] The term "vaccination" is employed in this article only in the sense in which it was primarily used by Jenner and Pasteur (immunization with the living, attenuated organism) and is not applied to forms of protective inoculation in which the killed organisms or their extracts are employed. See Kolle's previous remarks on this subject.

[6] *This Journal* (1906), 1, 181.

II. DISCUSSION OF THE PROPHYLACTICS PREVIOUSLY EMPLOYED.

Before proceeding directly to this discussion of the experimental work, it will be advantageous to mention the various prophylactics which have already been used in human beings, and briefly to review the methods of preparing them and the immunizing powers which they possess.

1. METHODS OF PREPARATION.

Haffkine's[7] prophylactic against plague consists of the killed bouillon cultures of the pest bacillus. The organism is grown in 3-liter flasks of bouillon upon the surface of which clarified butter or coconut oil is distributed. The cultures are kept for six weeks at a temperature between 25° and 30° C. The bacteria form stalactite-like growths, which extend from beneath the surface of the oil downwards. The flasks are shaken every few days, when the organisms fall to the bottom of the vessel and a new growth takes place above. After five or six weeks' growth, in order to be sure that the bouillon cultures are pure, subcultures are made from them upon agar slants. The bouillon cultures are then killed by heating for one hour at 65° to 70° C. and, after their sterility is proven, sufficient carbolic acid is added to form a 0.5 per cent solution. The prophylactic is placed in 30 cubic centimeter bottles and it must be shaken before use, since the killed bacteria naturally settle to the bottom of the vessel. The dose for men is from 3 to 3.5 cubic centimeters, for women from 2 to 2.5 cubic centimeters and for children from 0.5 to 1 cubic centimeter. Recently, Haffkine has in special cases recommended as high as 20 cubic centimeters for a single injection. He also frequently advises a second and even a third inoculation.

Another form of prophylactic against plague is that which was recommended by the German Plague Commission[8] (Gaffky, Pfeiffer, Sticker and Dieudonné) and which consists of the killed agar cultures of the virulent pest bacillus. Forty-eight hour agar slants of the organism are suspended in saline solution or bouillon and killed by heating for one hour at 65° C. Carbolic acid is then added to 0.5 per cent. The dose for a grown man is one agar culture. This prophylactic has many advantages over that recommended by Haffkine since with it an accurate dose as well a fixed virulence for the organism employed can be obtained. Moreover, it is much less dangerous, since either the tetanus bacillus or the organism of malignant œdema may develop in the bouillon cultures. Such anaërobic organisms will naturally not multiply on the agar slants.

Haffkine claimed as an advantage of his method that certain metabolic substances of the plague bacillus were contained in the fluid media which rendered the prophylactic more effective.

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[7] *Brit. Med. Journ.* (1897), 1461; also Lancet (1899), **77**, 1695.

[8] Bericht. der deutsch. Pest Kommission. *Arb. a. d. k. Gsndhtsamte*, Berl. (1899), **16**, 306.

However, both the German Plague Commission and Kolle have shown that the immunizing substances in Haffkine's prophylactic are mainly contained in the sediment consisting of the killed organisms. Kolle[9] showed that the clear fluid drawn off above the dead bacteria possessed but little immunizing power and further demonstrated that the number of bacteria in the old bouillon cultures was very small in proportion to the number that could be obtained in the same volume of fluid if agar cultures were used. Thus, one agar culture contained about as many bacteria as from 80 to 100 cubic centimeters of an old bouillon culture. Obviously whatever immunizing power the clear fluid above the sediment possesses is due to the free receptors which have been separated from the dead bacilli. It therefore seems evident that killed agar cultures are superior for inoculation to the killed bouillon ones.

Lustig and Galeotti in 1897[10] recommended the use of an extract of the plague bacillus obtained by chemical reagents. For its preparation three or four day agar cultures of the organism are thoroughly shaken with 1 per cent caustic potash solution.

After two hours, a 0.5 per cent acetic acid solution is slowly added until the mixture becomes slightly acid, when a flocculent precipitate forms. This sediment is collected on filter paper, washed to a neutral reaction and finally dried in vacuo. The powder when it is to be used is dissolved in 1 per cent sodium carbonate solution. The dose for man is from 2 to 3 milligrams. The Swiss serum institute in Bern recommends this prophylactic particularly because of the fact that an exact dose can be obtained and on account of the ease with which it can be transported.

Another protective against plague has been recommended by Terni and Bandi[11] in 1900. For the preparation of this prophylactic guinea pigs or rabbits are inoculated intraperitoneally with a small quantity of a suspension of the pest bacillus in bouillon. The animal usually dies in from thirty-six to forty-eight hours. At the time of its death, or a little before (in order to be sure that no migration into the peritoneal cavity of the intestinal bacteria has taken place) the abdominal cavity is opened, the peritoneal exudate collected and, if it is very thick, diluted with saline solution. The exudate is then placed in the incubator at 37° C. for twelve hours; it is next heated at from 50° to 52° C. for one hour on two successive days; in this way it becomes sterilized and the serum albumins present are not coagulated. A mixture of carbolic acid, 0.5 per cent, sodium carbonate 0.25 per cent, and sodium chloride 0.75 per cent is then added, in order to aid in the preservation and in the absorbability of the prophylactic when used. From 1.5 to 2.5 cubic centimeters are recommended for human injections.

The method of inoculation against plague recommended by Shiga[12] is as follows: The growth from a three-day agar culture which contained about 3 oesen of plague organisms was placed in a mortar with 3 cubic centimeters of salt solution and thoroughly rubbed up. The suspension was then heated for 30 minutes at 60° C. and later, carbolic acid to 0.5 per cent was mixed with it. After twenty-four hours an equal volume of a pest immune serum was added. Shiga recommended two injections; for the first one, 0.6 to 1 cubic centimeter of the mixture of serum and organisms and for the second, after the reaction had subsided, the same amount of the suspension of the killed organisms alone. He also advised a third inoculation with a larger dose for those who are frequently exposed to plague infection. The addition of the plague serum is recommended in order to diminish the local and general reaction and to aid in absorption.

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[9] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1901), **36**, 396.

[10] *Deutsche med. Wchnsch.* (1897), **23**, 227, 289.

[11] *Deutsche med. Wchnsch.* (1900), **26**, 463; also *Rev. d. Hyg.* Paris (1900), **22**, 62.

[12] Ber. über die Pest in Kobe und Osaka, Tokyo (1900), 54.

The prophylactic recommended by Besredka[13] in 1902 is prepared by adding a plague immune serum to a suspension of the killed pest bacillus in saline solution. By this means the bacteria become agglutinated and sink to the bottom of the vessel. The organisms are killed by heating for one hour at 60° C. The precipitate of bacteria is then washed thoroughly to free it from any excess of serum, and the agglutinated and washed bacteria are used for the injection. This prophylactic, Besredka maintained, produced an immunity in animals after forty-eight hours, which lasted for five months. Recently[14] he has recommended the addition of a normal horse's serum in place of the immune serum, to the suspensions of the killed and dried bacteria in saline solution. The organisms after separation by centrifugation are then said to be "atoxique" and are used for the inoculations.

Gosio[15] recently has described in detail a practical method of preparing plague prophylactic in large quantities. Pest bacilli were cultivated in thin layers of bouillon, in flasks such as are used for the preparation of diphtheria toxin. The bacteria were then precipitated by means of an agglutinating pest serum and separated from the latter; following this they were killed by heating at 65° C. for one hour, and the sterility of the mixture proved by the addition to the inoculated bouillon tubes of a small amount of potassium telluride.

Hueppe and Kikuchi[16] in October, 1905, announced that successful results had been obtained in guinea pigs by repeated inoculations of exudates containing plague aggressin. The experiments are very few in number and but briefly related, but the authors mention that in this paper it is their intention merely to call attention to the priority of the use of this method of immunization of animals against plague. As yet they have not reported further upon the subject.

In November, 1905, I for the first time reported to the Manila Medical Society upon vaccination in man with attenuated living cultures of the pest bacillus, and later, in February, 1906, published in this JOURNAL[17] and afterwards with Professor Kolle,[18] further experiments relating to this subject. The details of all the work performed in vaccination with these cultures will be considered in the present paper.

Finally, in January of this year (1906) Klein[19] has described a method of plague inoculation which he has employed in white mice, rats and guinea pigs. The buboes, spleen, lungs and liver of a guinea pig which has succumbed to subacute pest infection were removed and finely minced aseptically, spread out in thin layers in sterile glass dishes and dried over sulphuric acid at a temperature of 46° to 47° C. for three days. The exposure at this temperature devitalized all the pest bacilli in the organs and also prevented the growth and multiplication of other extraneous organisms. After three days of desiccation the dried scales of the material were rubbed down to a fine powder in a sterile mortar. The material was finally dried for from two to three days, at 37° C., in a wide-mouthed bottle.

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[13] *Ann. d. l'inst. Pasteur* (1902), **16**, 918.

[14] *Ann. d. l'inst. Pasteur* (1905), **19**, 479.

[15] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1905), **50**, 519.

[16] *Centrbl. f. Bakteriol. Orig.* (1905), **39**, 610.

[17] *This Journal* (1906), **1**, 181.

[18] *Deutsche Med. Wchnsch.* (1906), **32**, 413.

[19] "Preliminary Report to the Local Government Board on a New Plague Prophylactic," 1906, London, Darling and Son; also *Brit. Med. Journ.* (1906), 155.

When the prophylactic was to be used the desired amount was weighed out, well rubbed down in sterile warm distilled water and the turbid emulsion thus obtained injected subcutaneously.

This material is said to contain not only "acutely active toxin but also the dead bodies of all the *Bacilli pestis* originally present in large numbers in the necrotic organs (buboes, spleen, liver and lungs) with the addition probably of other substances of an undetermined nature and action."

Klein states that 10 to 15 milligrams of the dried powder confers immunity on the adult rat whereas 5 cubic centimeters of Haffkine's prophylactic, strongly turbid with flakes and masses of bacilli, does not do so, 10 cubic centimeters being necessary. The above prophylactic, in doses of 1 to 5 milligrams, killed a large percentage of mice within twenty to twenty-four hours and from 12 to 25 per cent of half-grown white rats in doses of from 5 to 8 milligrams, if the material was derived from acute cases. However, if obtained from the necrotic organs of guinea pigs dead of subacute plague (death in from five to nine days) as much as 10 to 12 milligrams were required to produce a fatal effect. Even as large amounts as 20 milligrams failed to kill a guinea pig of 200 to 300 grams weight. An adult rat weighing 120 to 200 grams, injected with 10 to 15 milligrams of the prophylactic of medium virulence, or with two doses of 10 milligrams each at an interval of nine to ten days, was given complete protection against even the most virulent *Bacillus pestis* when tested from one to thirteen weeks after immunization. A dose of 20 milligrams of the prophylactic in question in the case of guinea pigs, (which animals Klein regards as *less* susceptible to plague than white rats) twice injected at intervals of ten to fourteen days did not afford protection in more than 50 per cent of the animals, the remainder dying upon the subsequent injection of virulent plague material. However, the death of the animals was delayed several days and in the great majority of instances, they showed suppurating buboes. Klein argues that 10 cubic centimeters of Haffkine's prophylactic will protect an adult rat, an amount which according to the statistics in India and elsewhere represents at least double that required for the protection of an adult human being, so that 5 to 7 milligrams of his dried prophylactic would suffice as the dose for the human subject.

S. Wallannah[20] has also described a somewhat similar method of obtaining an extract from plague organs he proposes for the treatment of plague cases, arguing that the lesions are probably the centers where the pest antibodies are manufactured in great quantity.

Passive immunization with serum Yersin, Calmette and Borrell[21] will be considered later in this article. While this method is particularly efficacious for a short period of time after the inoculation, the protection afforded by it persists only for a few days. Eight days after the injection its protective action becomes weaker, and after twelve it is almost entirely lost.

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[20] *Centrbl. f. Bakteriol. Orig.* (1906), **42**, 471, and *Lancet* (1907), **172**, 222.

[21] *Ann. d. l'inst. Pasteur* (1895) **9**, 589.

2. CONSIDERATION OF THE EXTENT EMPLOYED IN MAN AND THE RESULTS OBTAINED.

The prophylactics described above constitute the methods of immunization against plague which have been recommended from the beginning of the nineteenth century up to the present time.[22] In regard to the extent to which they have been used in man it may be said that Besredka inoculated himself with his prophylactic but, so far as I am aware, it has not been further employed in human beings. The methods of Gosio, Hueppe and Kikuchi and Klein have not, to judge from the absence of reports, as yet been used in man. Forty-seven persons were inoculated by Shiga's method in 1899 in Kobe and Osaka. The Terni-Bandi method was employed in Rio de Janeiro, Brazil, in the epidemic of 1889 to 1901. According to Havelburg several hundred people were inoculated in that city and no cases of plague occurred among them, with one exception, where the individual sickened on the day of inoculation and where the attack of pest was mild and resulted in the recovery of the patient. The inhabitnats of Rio de Janeiro number about 750,000, and only 589 cases of plague occurred in the entire city; therefore from these human statistics, we can not form a judgment of the value of the method. Dessy in the plague epidemic in 1900, in San Nicola, La Plata, inoculated 200 persons with Lustig's prophylactic. None of the vaccinated sickened with pest. The same remark applies to these statistics as to the ones collected from the cases in which the Terni-Bandi method was employed. The prophylactic recommended by the German Plague Commission has also not been very extensively employed in human beings, although Zupitza has used the method to some extent in East Africa. Over 127,000 cases in Japan have been inoculated with small quantities (2 milligrams to each case) of killed agar culture of the pest bacillus.

The protective which has obviously most widely been used in man is that recommended by Haffkine, and this I believe chiefly to be due to the fact that Haffkine has resided in India and has had abundant opportunity to employ his method. It is not my purpose here to enter into a discussion of the available statistics of the results of the human inoculations performed in India. They are to be found in the publications of the Indian Plague Commission, in other Indian government reports, and in Haffkine's very recent article on the subject.[23] From their study one becomes convinced that it is very difficult to decide just what the value of the inoculations performed by this method has been. However, it seems unquestionable that large and repeated doses of Haffkine's prophylactic frequently protect individuals against plague infection, although it is well known that many of the inoculated persons, including even a number of those who received more than one injection of the prophylactic, have contracted the disease. Thus, the Indian Plague Commission found that some individuals who during two years had received four injections of the prophylactic, fell victims to pest; and, on the other hand, as many as 8 per cent of the inoculated in one district, Bulsar, contracted the disease. In 1902 it was the intention of the Indian government to inoculate the inhabitants of a province containing about six millions of people with this prophylactic. However, the work was discontinued owing to 18 deaths from tetanus which occurred among the inoculated shorty after the project was undertaken.

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[22] In the eighteenth century some desultory attempts were made to secure immunity in man by exposing the individual to direct infection with plague pus. These methods were soon abandoned owing to the disastrous results following their employment. I have previously considered them elsewhere. *This Journal* (1906) 1, 181.

[23] *Bull. de l'inst. Pasteur* (1906), 4, 825.

It is important in discussing the value of Haffkine's inoculations to consider the work of Kolle and Otto[24] on the immunization of guinea pigs with Haffkine's prophylactic. These authors conclusively showed that guinea pigs could not, except in rare instances, be immunized against pest infection by the use of this prophylactic and their experiments led them to emphasize the fact that if large and repeated doses of the killed pest organism failed to immunize such small animals as guinea pigs, it seemed unreasonable to expect very favorable results in man from such a method, particularly since the amount of the bacteria inoculated in human beings is so much smaller in proportion to the body weight.

However, the subject with which I was at first most concerned was work which would lead to a decision as to the *most effective* method of inoculation against pest and this it seemed could best be carried out by experimental studies on animals.

It therefore was from this standpoint that I decided first to investigate the subject.

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[24] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1903), **45**,

III. CULTURES EMPLOYED IN THE EXPERIMENTAL WORK.

The cultures of *Bacillus pestis* employed in the experimental work considered in this article consist of one virulent and three avirulent strains of the organism, which will be designated in the descriptions as "Pest Virulent," "Pest Avirulent," "Maassen Alt," and "Avirulent Manila."

"Pest Virulent."—The strain "Pest Virulent" was obtained from a human case of plague which succumbed in October, 1905. It was isolated from the spleen, at autopsy, by plate cultures and was then grown upon an agar slant for one generation. From this culture it was inoculated into a guinea pig. Upon the death of this animal, a portion of its spleen was rubbed over a freshly shaved area on the abdomen of the second guinea pig and this process of inoculation from the spleen of one animal to the abdomen of a fresh one has successively been repeated from animal to animal without an interruption for more than a year and for over two hundred passages of the strain. (See Series of inoculations, p. 296.) Whenever it was desirable to experiment with this strain, cultures were made upon agar from the heart's blood of one of this series of guinea pigs on the day of the death of the animal, and twenty-four hours later the growth, if pure, was transferred to a second agar tube. A second transfer of this culture (never removed longer than three or four days and usually not longer than seventy-two hours from the animal body) was used in my experiments in testing the immunity of the animals inoculated by the different methods, excepting in a few instances in which a first transfer was employed; in these cases it is so stated in the description of the experiments. Hence, the method of testing was uniform, a 48-hour slant agar culture of the first or second transfer invariably being employed. In the preparation of all the prophylactics with which I experimented, in the manufacture of which it was desired to use a virulent organism, a first or second transfer of this culture was also employed. The strain "Pest Virulent" kills guinea pigs, usually in from three to seven days after cutaneous inoculation by massaging the shaved abdomen of the animal with a portion of a plague spleen from another one (see p. 296), and hence it represents a very virulent strain of *Bacillus pestis*. It may be seen from the experiments which will later be recorded in detail that, during the entire time of its passage through guinea pigs, the virulence of this strain for these animals and for rats and monkeys re-

mained unchanged. It is perhaps needless to add that its morphology and cultural characteristics are typical of other unquestionable strains of *Bacillus pestis* and that it is agglutinated by a standard pest serum. A small standard oese was employed throughout the work, of such a caliber that one 48-hour second-generation culture of this strain yielded about 10 oesen of growth.

"Pest Avirulent."—This strain was obtained through the kindness of Prof. W. Kolle, at present Director of the *Institut für Infektionskrankheiten,* in Bern. It represents an attenuated strain of "Maassen Alt," presently to be described. It forms colonies on agar typical of other strains of *Bacillus pestis* and in other ordinary media its growth resembles this organism. Its morphology on agar, while not perfectly typical of *Bacillus pestis,* is suggestive of this organism, since a number of bipolar staining bacilli may be distinguished in the cover slips made from the fresh culture.

A 19-hour agar slant culture of this organism was suspended in bouillon and inoculated beneath the skin of the shaved abdomen of a monkey. Six hours later an incision was made near the point of inoculation and cover slip preparations and cultures secured from the drops of blood which escaped from the incision. No bacilli were found in the cover slips, but the cultures developed numerous colonies presenting the typical appearance of those of *Bacillus pestis.* Microscopical preparations from these colonies revealed plump bacilli, a few involution forms, and others with typical bipolar staining. Some of the organisms occurred singly, others in pairs or rarely in chains of three or four. Some of them appeared encapsulated. In other instances also in which very large amounts of this strain were inoculated intraperitoneally into guinea pigs and in which the animal succumbed to pest intoxication, organisms with the typical morphology of *B. pestis* have also been observed in microscopical preparations made from the abdominal cavity. (See Series 32.) The strain "Pest Avirulent" is agglutinated by two standard pest sera. Its virulence is so reduced that from 1 to 2 whole agar slant cultures do not cause the death of guinea pigs when injected subcutaneously. In small animals under 175 grams in weight, one agar slant injected *intraperitoneally* has occasionally caused death from plague *intoxication.* (See Series 37, animal, number 2043, p. 207.) The unquestionable proof that the organism really represents an attenuated strain of the genuine pest bacillus is furnished by the fact that monkeys and guinea pigs vaccinated with this culture have later been shown to possess high and undoubted pest immunity, by subsequently inoculating them with multiple lethal doses of the strain "Pest Virulent" already described. (See Series 4, 11, 12, 18, etc., pp. 199 to 212.)

Pest "Maassen Alt."—This strain was also obtained by me from Professor Kolle, who originally received it from Dr. Maassen. Both it and the previous strain have been described by Kolle and Otto,[25] and extensively employed by them in the immunization of various laboratory animals. The cultural peculiarities and morphology of pest "Maassen Alt" are typical of *Bacillus pestis* and microscopical preparations from its cultures show the characteristic bipolar staining of the bacilli. Such forms are particularly noticeable after the bacillus has been inoculated into the subcutaneous tissues of an animal and several hours later reclaimed in cultures. This organism is also agglutinated by two standard pest sera; it likewise represents a much attenuated strain of *Bacillus pestis,* although its virulence is not so far reduced as is that of the strain "Pest Avirulent." Guinea pigs of from 200 to 300 grams weight as a rule recover from subcutaneous injections of such large amounts as from one to two agar slant cultures, yet they sometimes succumb to such inoculations. Evidences of a subacute pest infection are visible in the event of their death. In other experiments, when as much as a whole agar slant culture has been injected intraperitoneally, or more rarely subcutaneously, the animal has died within twenty-four to forty-eight hours, apparently of a toxæmia, the organisms not having multipled to any demonstrable extent, or at least they have certainly not invaded the circulation or the solid organs. This has been demonstrated by the sterility of cultures made from these locations. (See Series 38, animal number 2050, p. 219, and Series 40, animal number 2128, etc., p. 220.) In still other instances in which the guinea pigs have lived six or more days after inoculation and then succumbed and in which the injection has been made subcutaneously, pest bacilli may be demonstrated in cultures from the buboes which have developed but they may not be encountered in the blood of the heart or any of the other organs of the body. As in the case of the strain "Pest Avirulent," undoubted proof that the culture "Pest Maassen" represents an attenuated type of *Bacillus pestis* has been given by the fact that numerous guinea pigs and monkeys have been vaccinated with it and have later shown high and undoubted immunity against inoculations with the strain "Pest Virulent." (See Series 17, 21, etc., pp. 212 to 221.)

Pest "Avirulent Manila."—The culture "Avirulent Manila" was obtained from an autopsy upon a typical case of human bubonic plague occurring in Manila in the autumn of 1903. Its exact virulence at the time of its isolation was not known. It was preserved in the laboratory as a stock laboratory plague culture by Mr. Hare, formerly of this Institute, and was transplanted from time to time on agar. It had not been passed through animals since its isolation up to the time these experiments were begun. In the summer of the year 1905, from 1 to 2 oesen of this culture constituted a lethal dose for guinea pigs of 250 to 300 grams weight, when inoculated subcutaneously, the animals usually dying from subacute plague infection. The virulence of this organism has still further been reduced by growing it at a temperature of from 41° to 43° C. in flasks of alcoholic bouillon for three weeks at a time as recommended by Hetsch.[26] (See p. 310, "Virulence of Pest Bacillus.") By this means a considerable reduction in virulence has occurred, since at the present time guinea pigs of 250 to 300 grams weight are usually able to withstand the subcutaneous inoculation of one entire 24-hour agar slant culture of this organism. However, occasionally they succumb to pest infection from a such dose, although the course of the disease in these instances is always prolonged. This organism also shows the typical morphology of *Bacillus pestis* and agglutinates with a standard pest serum; animals vaccinated with it also acquire pest immunity. As this strain was known formerly to have possessed a greater virulence in nature and had been artificially attenuated, experiments were performed with it in monkeys to ascertain whether it would be possible to cause it to regain its original virulence. However, from this standpoint these experiments were unsuccessful. (See p. 301.)

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[25] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1903), **45**, 513.

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[26] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1904), **48**, 442.

IV. ANIMALS EMPLOYED—THEIR SUSCEPTIBILITY TO PEST INFECTION—LETHAL DOSE—TECHNIQUE OF INFECTION.

Rats and monkeys, in addition to guinea pigs and rabbits, mainly have been employed in the experimental work.

Rats.—The rats used in the majority of cases were the ordinary wild ones found in Manila belonging to the species *Mus decumanus,* and in a few instances to the species *M. rattus.* They contract pest infection naturally and have been known to be concerned in the spread of plague in Manila since 1902. During that year the pest bacillus was found in the organs of 0.34 per cent and in the following year in 0.0625 per cent of these rodents collected from various portions of the city of Manila and sent by the Board of Health to the laboratory for examination. The majority of the rats so infected had been found dead or were captured in the houses where cases of human plague had occurred. Since the decline of plague in Manila, only an occasional rat has been found to be infected. Wherry reported from this laboratory one such instance in 1905, and during the present year (1906) the pest bacillus has been isolated but twice from rats collected in Manila and sent to the laboratory for examination. These wild rats could be obtained alive in great abundance and on account of the frequent scarcity and the difficulty of breeding white rats here in this city, the wild species was used in the majority of the plague experiments and they proved very satisfactory animals. The rats were always kept for some time after their capture before being employed for experimental purposes. They were satisfactorily handled during the inoculations by two Filipino assistants, who protected themselves from being bitten by wearing extra heavy leather gloves. Rat tongs or pincers were not employed because of the danger of traumatic injuries to the animals. As was expected, the species *M. decumanus* proved itself to be readily susceptible to plague infection. In the experimental subcutaneous inoculations of these rats a second transfer of a 48-hour culture of "Pest Virulent" was suspended in 5 cubic centimeters of bouillon or saline solution and a syringe needle from a 5 cubic centimeter syringe dipped in this suspension and then thrust beneath the skin near the base of the tail of the rat. After such an inoculation the animal almost invariably succumbed to pest infection, usually in from three and one-half to five days, although life sometimes was prolonged to from the seventh to the fifteenth day after inoculation.

A single animal which evidently possessed unusual resistance or acquired immunity, lived for twenty-two days and finally died of chronic pest.

Chronic forms of pest, such as have been described by Kolle and Martini,[27] in which the inoculated rats lived for months, have not been observed in any of the animals injected with the suspension of the strain "Pest Virulent" in the doses mentioned above. It seems not unlikely that less virulent strains of the organism have usually given rise to the chronic infections in rats. Whenever the death of the rat occurred from the infection, pest bacilli were always found at autopsy in the tissues near the point of inoculation and almost always in the spleen. However, in the spleen the organisms were frequently not so numerous as near the point of inoculation, and in the heart's blood, while they usually were present in abundance, sometimes they were found only in small numbers and occasionally they even were absent.

The Japanese variety of white rats which was used was probably the albino variety of *Mus rattus*. They also almost invariably succumbed to pest infection in a manner similar to the wild rats and they seemed to be equally or somewhat more susceptible to the same amount of the strain "Pest Virulent" than the species of wild rat (*Mus decumanus*).

Whether the Manila wild rat through its progenitors has gradually acquired a slightly greater insusceptibility to pest, a disease to which it has been exposed from time to time, can not be stated. Albrecht and Gohn found gray rats slightly more susceptible than white ones, although the experiments upon which they based their conclusions were not numerous.

Monkeys.—The study of pest infection in monkeys is particularly important, since these animals suffer with forms of the infection analogous to those seen in man and, moreover, are said sometimes to contract the disease naturally. I have never observed a case of spontaneous infection in a monkey in Manila, but I have never systematically sought for such an infection.

In the report of the Indian Plague Commission there is some evidence of the occurrence of plague in monkeys. Most of it is not entirely conclusive, but in some instances the diagnosis was confirmed by bacteriological examination. Albrecht and Gohn[28] have also mentioned spontaneous infection in these animals and Simond[29] and Clemow[30] have reported epidemics among them.

The latter author on three occasions observed that monkeys which were supposed to be of the species *Macacus synicus*, sickened spontaneously and died. Plague bacilli were isolated from a number of the animals at autopsy.

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[27] *Deutsche med. Wchnschr.* (1902), **28**, 3.

[28] Uber die Beulenpest in Bombay: *Denkschr. d. math.-naturw. Klasse d. Kais. Akad.*, Wien (1898–1900), **66**, 726.

[29] *Ann. de l'inst. Pasteur* (1898), **12**, 664.

[30] *Brit. Med. Journn.* (1900), 1141.

Statements in the literature in regard to the lethal dose of pest bacilli for monkeys of different species vary somewhat and are not entirely definite.

Albrecht and Gohn[31] (Report of the Austrian Plague Commission) in their experiments with monkeys used the small, long-tailed, brown Indian species. Inoculations of living cultures in amounts of from 0.5 to 4 oesen were made. With some strains 0.5 oese killed animals after forty-eight hours and with others 2 oesen failed to kill. It is not possible from their experiments to arrive at a general determination of a lethal dose for these animals, since strains of different virulence and in different amounts and with different methods of inoculation were employed and no series of animals by any one method of experimentation is given.

Wyssokowitz and Zabolotny[32] (Russian Plague Commission) in their experiments used three Indian species, described as the *Macacus* with the long tail, the *Macacus* with the short tail and the black ape with a long tail. The two latter were found to be more susceptible to pest infection, dying in from two and one-half to three days, while the former usually succumbed in from four to five days after inoculation. These monkeys proved to be very sensitive to small amounts of plague bacilli. A prick on the palm of the hand or sole of the foot of the animal with a needle which had been moistened with a plague culture, invariably produced death in from three to ten days.

In 1901 Zabolotny,[33] in further experiments, confirmed the fact that in the animals of both the species *Macacus radiatus* and *Semnopithecus entellus* a small amount of pest bacilli gave rise to fatal infection, but that the latter species was more susceptible and succumbed in a shorter time.

The German Plague Commission used two varieties of monkeys in their experiments, *Macacus radiatus* and *Semnopithecus entellus.* The later species was extraordinarily sensitive to pest infection, succumbing in six days to the subcutaneous injection of 0.01 or 0.001 oese of the virulent plague bacillus and dying of larger inoculations after two days. These monkeys also succumbed to pest infection if small amounts of pest bouillon cultures were rubbed into superficial wounds of the skin. The species *Macacus radiatus* was much less susceptible. When the skin was scarified and a fresh agar culture rubbed over the wounded area, or 0.01 of an oese of an agar culture injected subcutaneously, the animals acquired a moderate pest infection, with fever, glandular swellings, etc., but usually survived. However, 1 oese of a two-day agar culture suspended in 1 cubic centimeter of bouillon and injected subcutaneously, always caused the death of this species in three or four days, and ½ or ¼ of an oese always brought about the same result, although in a slightly longer time.

In the report of the Indian Plague Commission mention is merely made of the fact that the gray Indian monkey is more susceptible to plague than the brown.

Only the common Philippine species (*Cynomolgus philippinensis* Geoff.) was employed in my experiments with monkeys. The similarity of pest infection in these monkeys to that observed in human beings is very marked, and in the experimental work with them all forms of infection with plague have been encountered. This species of monkey appears to stand between *Macacus radiatus* Geoff. (*Macacus sinicus* Linn) and

[31] *Loc. cit.*, 713.

[32] *Ann. d. l'inst. Pasteur* (1897), **11**, 663.

[33] *Arch. Sci. Biologiques* (1901), **8**, 390.

Semnopithecus entellus Cuvier in relation to susceptibility to plague infection. However, since all three of these species apparently belong to different genera, it is perhaps not strange that their susceptibility to the infection should vary to a certain extent.

Obviously, in the beginning of this work it was important to determine the lethal dose with a pest culture of known virulence for the species *Cynomolgus philippinensis* with which the experiments were to be performed and this was done as accurately as possible. However, the susceptibility of the individual monkeys of this same species was found to vary considerably. Usually, the animal dies from pest infection in from three to seven days if the growth from a 48-hour agar slant culture of the strain "Pest Virulent" is suspended in 5 cubic centimeters of bouillon, and a 5 or 10 cubic centimeter syringe needle dipped in this suspension and then thrust beneath the skin near the root of the tail. Also, if the skin is shaved over a small area and slightly scarified and then a suspension in bouillon of the same culture is rubbed over this area, the animal usually succumbs. On the other hand, some of these monkeys will survive the inoculation of similar and of even much larger amounts of the virulent pest organism, certain of them remaining alive after the injection of even ¼ and ⅓ oese of "Pest Virulent". However, it has been found that ½ oese of "Pest Virulent" suspended in 0.25 cubic centimeter of bouillon always constitutes a fatal dose for monkeys averaging about 2,000 to 3,000 grams in weight, and therefore, although usually this quantity of plague culture really represents many times the multiple lethal dose for the majority of these animals, it has been employed in testing the immunity of all the monkeys used in my experiments and weighing under 3,000 grams, excepting in the first series of experiments where the lethal dose had not been determined accurately and where it is then noted in the tables. For monkeys over this weight ⅔ oese of "Pest Virulent" suspended in 0.33 cubic centimeter of bouillon has been employed. In order that the size of dose used in testing all the animals might be as uniform as possible one 48-hour agar slant culture of the strain "Pest Virulent" was suspended in 5 cubic centimeters of bouillon or saline solution; 0.25 cubic centimeter of the suspension then contains ½ oese and 0.33 cubic centimeter ⅔ oese of this organism.

The variations in susceptibility between different individuals of this species of monkey are evidently of considerable importance in the study of their immunization and, as will be seen from the experiments which will be related further on, this individual variation probably accounts largely for the different results in immunization which have been obtained in animals of the same weight and inoculated with the same dose and by the same method.

If, for example, each animal of a series is inoculated with a fixed amount of plague vaccine and, later, the immunity of each is tested by the inoculation of equal amounts of the virulent pest bacillus, if the susceptibility of the different animals varies, then different results in mortality must occur both for the reason that different degrees of immunity will arise in the different animals from the primary inoculation of the same dose of the vaccine and for the reason that in testing the immunity, the fixed amount of the virulent organism employed will represent in the more susceptible animals a greater multiple of the lethal dose than it does in the less susceptible ones. This variation in susceptibility to the action of the plague bacillus has been found to be much more marked in the monkeys I have used than it is with other laboratory animals, and it seems not unlikely that the conditions relating to susceptibility in these animals approach nearer to those which exist in man than they do to those which are present in such animals as guinea pigs, rats, mice, etc. During the past year we have had numerous opportunities to observe the differences in immunity obtained in different human beings by the inoculation of the same sized dose of our cholera prophylactic, by studying the blood serum from individuals vaccinated against Asiatic cholera. These variations in some instances have been very decided and have been much greater than those which have been observed in series of guinea pigs or of rabbits, all inoculated with an equal dose. Hence, the limit of value of a method for the immunization of man against plague can probably better be studied in monkeys than in any of the other lower animals. It is particularly for this reason and because of the fact that monkeys suffer with forms of plague analogous to those observed in human beings, that they have been extensively used in my experiments in testing the final value of methods of pest inoculation which have proved effective in the ordinary laboratory animals. Moreover, another reason for the extensive use of monkeys has been that it might be argued that the value of a method of immunization against plague in man should not be judged by its action in experiments upon such animals as guinea pigs and mice alone, an argument which has already been advanced.

Guinea pigs and rabbits.—Two methods of infection were employed in guinea pigs, for the purpose of testing their immunity following the prophylactic injections in the various experiments. The first consisted of the suspension of a 48-hour agar culture of the strain "Pest Virulent" in 5 cubic centimeters of bouillon. Five large oesen of this suspension were then rubbed over a shaved area of the abdomen of the animal and the skin scarified with a scalpel. The other method of infection less commonly employed consisted of massaging over a shaved area of the abdomen of the guinea pig a portion of the spleen of a second one just dead from acute pest infection with the strain "Pest Virulent." By either of these methods the guinea pig, unless previously immunized, invariably succumbed to acute infection. Rabbits were only employed in certain of the agglutinative experiments and in the preparation of aggressin exudates, under which subjects the technique of the inoculations is described.

In testing the immunity of all the animals care was taken to introduce the infection upon the opposite side of the body to that upon which the vaccination or prophylactic injection had first been made. This precaution was taken in order to avoid any chance of obtaining results which might have been influenced by the presence of a local immunity which had developed in the animal, particularly in the tissues about the point where the first inoculation had been made. Wassermann and Citron[34] have recently called special attention to the local development of immune bodies.

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[34] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1905), **53**, 331.

V. IMMUNIZATION OF ANIMALS.

1. WITH KILLED PEST BACILLI.

Yersin, Calmette and Borrel[35] in 1895 first called attention to the fact that rabbits, inoculated subcutaneously on three or four occasions with gelatine cultures of the pest bacillus killed by heating for one hour at 58° C., were rendered immune to subsequent subcutaneous inoculations of virulent plague bacilli. Guinea pigs were much more difficult to immunize, and they rarely succeeded in thoroughly protecting one of these animals by such a method.

Wyssokowitz and Zabolotny[36] mention that monkeys can be immunized against pest by the inoculation of killed cultures of the organism, although they give no details showing the percentage of animals that were protected by the use of this method.

The German Plague Commission[37] found that all the monkeys of the species *Macacus radiatus* which received subcutaneously one carefully killed, two-day culture of the virulent pest organism were able later to resist almost without reaction the subcutaneous injection of one full oese of the living culture. However, with this same amount they were not able to immunize gray monkeys of the species *Semnopithecus entellus* against plague infection, and the Commission did not have time to pursue the question further with this species of animal. However, they demonstrated in a series of rat inoculations that a large percentage of the animals could be immunized with killed cultures of the pest organism against subsequent subcutaneous infection, if the dose was sufficiently large (2 killed agar cultures). One agar culture did not suffice to immunize the animal. The rats often succumbed from the effect of the large dose of the primary inoculation.

Albrecht and Gohn[37] state that they were able to immunize guinea pigs by repeated doses of killed agar cultures of the pest bacillus, although the immunity obtained was not great. In their experiments with these animals, described in their report, the guinea pigs all finally died of pest infection.

Tavel, Krumbein and Glucksmann[38] were able to obtain immunity in some instances in the course of a small number of experiments with rats, by the inoculation of large amounts of the killed cultures, but they were unable in many experiments to immunize a single guinea pig even by using repeated inoculations of the killed cultures.

Beinarowitch[39] concluded that the injection of killed cultures of the pest bacillus conferred an immunity upon rodents, but that this was very slight, unless the inoculation was repeated several times.

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[35] *Ann. d. l'inst. Pasteur* (1895), **9**, 589.

[36] *Ibid* (1897), **11**, 667.

[37] *Loc. cit.*

[38] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1902), **40**, 239.

[39] *Arch. d. Sci. Biologiques* (1903), **9**, 343.

The most important and most convincing experiments in regard to the value of immunization with killed cultures of the plague bacillus in rats and guinea pigs have been made by Kolle and Otto.[40] In their experiments the loss *during the process of immunization* of the rats with Haffkine's prophylactic was 38.5 per cent and with the killed agar cultures 33.3 per cent.

Later, on testing the immunity of the animals remaining alive, it was found that only 21.9 per cent of those which had been inoculated with the killed agar cultures, and but 22.2 per cent of those inoculated with Haffkine's prophylactic, gave evidence of an acquired immunity by surviving the test. Attempts were made to immunize 26 guinea pigs with killed agar cultures, amounts as large as from ½ to 1 entire agar culture being injected subcutaneously. During the process of immunization four of the animals died. Of the remaining twenty-two, only two (7.7 per cent of the whole) appeared to be immune on subsequent testing. Hardly more favorable results were obtained in the experiments in which killed bouillon cultures were employed. Twenty animals were inoculated with Haffkine's prophylactic. Two of these died from the effects of the immunization and of the remaining eighteen, only two (10 per cent) remained alive after reinoculation with the virulent organism. Other experiments on guinea pigs were also performed in which repeated inoculations were made with killed cultures of the plague organism. The animals were first injected with 1, then with 1½ and finally with 2 killed agar cultures or with 1, 1½ and 3 cubic centimeters of Haffkine's prophylactic. In the process of immunizing twenty guinea pigs by these methods, six of the animals died from the effects of such large doses of the killed bacteria. The immunity of the remaining fourteen was tested six weeks after the last injection with the living virulent plague bacillus when only one animal remained alive and proved to be immune.

In my own experiments the value of inoculation with killed cultures of the pest bacillus was tested on monkeys and guinea pigs.

ANIMAL EXPERIMENTS.

A. With monkeys.—It seemed desirable to experiment further with killed cultures of the pest bacillus for two reasons, first, to compare the immunizing value of the dead organism with that of other methods of inoculation, such as those of vaccination, natural and artificial aggressin injections etc., and second to see whether sufficiently good results could be obtained by experiments on Manila monkeys to warrant advocating the use of this method in man.

In Series 5, page 189 are given the results of the experiments on eight monkeys inoculated with killed bouillon cultures (Haffkine's method) in varying amounts. On testing the immunity of these animals by thrusting a syringe needle infected with virulent pest bacilli beneath the skin, eleven days after the primary inoculation, only three were found to possess sufficient immunity to survive the infection. Of those which died, one had previously received 30 cubic centimeters and another 20 cubic centimeters of the prophylactic. From what has been said under the discussion of the susceptibility of this species of monkey to pest infection, it will be seen that the method of testing the immunity of the animals employed in this series was not severe, since in some instances, monkeys which had not been protected at all, survived such a method of infection (for an example of this fact see Series 16, p. 188, animals numbered 1357 and 1358). However, it is true that the two control animals inoculated in Series 5 died of pest infection. In Series 49 (p. 197), nine other monkeys were inoculated by the same method, from 10 to 15 cubic centimeters being injected in each instance. One month after the inoculation, the immunity of the animals was tested and only two were found to resist the infection (numbers 2699 and 2703).

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[40] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1903), **45**, 512; (1904), **48**, 399.

Because of the superiority of the killed agar cultures over the killed bouillon ones, the reasons for which have already been pointed out, only the former were employed in the remainder of the experiments of this nature.

Three monkeys in Series 9 (p. 190) were inoculated, each with two 48-hour killed agar cultures of the strain "Pest Virulent." Ten days later, upon the injection of 2 oesen of the living virulent organism, all the animals succumbed to the resulting infection. The amount used in testing the immunity of these animals obviously was excessively large, as was shown by later experiments; therefore, the series does not represent a fair test of the value of this method of immunization.

The experiments recorded in Series 23 (p. 191) comprise 18 monkeys besides the control animals. The pest organisms in the suspension were not all killed by heating before inoculation, and hence no accurate conclusions can be drawn from these experiments in regard to the immunity produced by the killed organism alone. The size of the dose used in testing the immunity of the animals was also not sufficiently large. (See remark under Series 23.)

In Series 25 (p. 192), twenty monkeys were inoculated with from one to two 48-hour agar cultures of the virulent, killed organism. Three of the animals succumbed from the effects of this inoculation. The immunity of the remaining seventeen was tested eighteen days after the primary inoculation, when but four (23 per cent) resisted the infection and remained alive.

In Series 48 (p. 195), fifteen monkeys were inoculated with one 48-hour agar slant of the killed virulent strain. One of these animals succumbed, its death being apparently caused by this inoculation. The immunity of the remaining fourteen was tested one month after the first injection when but four, or 28 per cent, remained alive.

B. With guinea pigs.—In Series 50 (p. 197), fifteen guinea pigs were inoculated, each with one 48-hour agar culture of the killed virulent organism. Upon testing the immunity of these animals one month later, but four, or 26 per cent, resisted the infection and remained alive.

No further attempts to immunize larger series of guinea pigs were made with killed cultures since, reasoning from the experiments of other observers as well as from my own, it appears that it is possible to immunize only a small percentage of these animals by such a process. The guinea pigs comprising Series 50 were inoculated with the killed agar cultures, chiefly for the purpose of comparing this method with the other methods of inoculation which were being tested at the same time.

In all, fifteen guinea pigs and seventy-three monkeys were inoculated with killed cultures of the pest bacillus; 26 per cent of the guinea pigs and 32 per cent of the monkeys subsequently proved to be immune. However, if Series 23 is excluded as it should be, since probably both killed and living organisms were used in the immunization of the animals, the percentage of immunity in the remaining monkeys is but 25 per cent.

2. INOCULATIONS IN ANIMALS WITH LIVING ATTENUATED CULTURES (VACCINATION).

I have recently[41] reviewed the literature regarding the early experiments made to attenuate virulent strains of the pest bacillus and of the inoculations performed in animals with other avirulent strains of this organism, and it is not my intention here to enter into any lengthy discussion of this subject but merely to mention these experiments.

The German Plague Commission made some attempts at attenuating strains of the pest bacillus for use in immunization, by exposing the living cultures both to a temperature of 51° C. for varying periods of time and also to the action of carbolic acid. These experiments resulted unsuccessfully, the organisms retaining their full virulence. Albrecht and Gohn[42] and Yersin and Carré[43] also performed experiments on guinea pigs, rats and monkeys with somewhat attenuated pest cultures. No extensive or convincing experiments in regard to the value of the living, attenuated cultures in the immunization of animals against plague had apparently been undertaken until Kolle and Otto investigated this subject. These authors, in numerous and careful experiments on rats and guinea pigs, showed that cultures of the pest bacillus so attenuated that they were no longer dangerous to these animals even in large amounts (2 agar cultures) were capable upon injection of giving rise in them to a much higher immunity than was produced by the killed cultures of the organism. Thus, in one series of fifty-nine guinea pigs which were immunized by a single inoculation of an attenuated living culture, thirteen died and two were killed for control purposes. On testing the immunity of the remaining forty-four from three to eight months after their vaccination, twenty-eight, or 63.6 per cent, remained alive.

In another series of thirty-four guinea pigs immunized with an attenuated pest culture (Maassen V), of which one died during the process of immunization, twenty-one were reinoculated with the virulent organism from one to four months after their vaccination, and of this number, sixteen (76 per cent) remained alive and five died. Nine other guinea pigs were inoculated with this agar culture and at the same time with plague immune serum. All proved to be immune upon reinoculation with the virulent pest bacillus.

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[41] *This Journal* (1906), **1**, 182.

[42] *Loc. cit.*

[43] Congress International de Méd. Section de Méd. et Chirug. Militaires. Sous section Coloniale Paris (1904), 54.

ANIMAL EXPERIMENTS.

As with the killed pest organism, monkeys and guinea pigs were employed in my experiments on animals with attenuated cultures of the pest bacillus.

Experiments on the immunization of monkeys with the strain "Pest Avirulent."—Six monkeys in Series 4 (p. 199) were inoculated subcutaneously, each with one 24-hour agar culture of the strain "Pest Avirulent." The immunity of the animals was tested ten days later, four being inoculated by thrusting beneath the skin a needle infected with virulent pest bacilli and two (numbers 1232 and 1233) by lightly scarifying the abdomen with a scalpel infected with this same organism. Since two control animals (numbers 1286 and 1287) also inoculated at this time in the latter way did not die, the monkeys numbers 1232 and 1233 can not necessarily be considered immune. Three of the four vaccinated animals wounded with the infected needle survived, while but one of four controls inoculated in the same manner recovered. The conditions encountered in this series are somewhat analogous to those met with in Series 5 (p. 189), where the killed organisms were employed.

Five monkeys in Series 11 (p. 200) were vaccinated, each with one 24-hour agar culture of "Pest Avirulent" and eleven days later their immunity was tested by the inoculation of 2 oesen of "Pest Virulent." Four of the animals died and one survived. The course of the disease in three of those which died was prolonged to twice the length of that in the control animals. In two (numbers 1277 and 1280) the blood serum had evidently acquired considerable anti-infectious power against the pest bacillus; this was presumed to be the case from the distribution of the bacteria at autopsy and from the fact that in one instance the heart's blood was sterile and in the other but six colonies of this organism developed in cultures from the heart's blood. Hence, these animals probably died rather from pest toxæmia than of septicæmia. Their blood may have acquired considerable anti-infectious power with but little antitoxic action. Obviously, the dose (2 oesen) employed in testing the immunity was exceedingly large. This series of experiments may be compared with Series 9 (p. 190), in which the animals immunized with dead bacilli all succumbed upon reinoculation with 2 oesen of the virulent strain.

In Series 12 (p. 201), nine monkeys were inoculated subcutaneously with from one to two 24-hour agar cultures of the strain "Pest Avirulent." One of the animals (number 1299) died twelve hours after the vaccination, of a streptococcus and staphylococcus infection which had existed prior to the vaccination. The immunity of the remaining eight was tested ten days after the vaccination, either by the inoculation of $\frac{1}{2}$ or of 1 oese of the strain "Pest Virulent." Four of the animals remained alive and well, and four died. In those which died, the course of the infection was greatly prolonged beyond that in the control monkeys and the pest bacilli had not as a rule invaded the circulation and other organs to the same extent as they had in the control animals.

Ten monkeys in Series 18 (p. 202) were vaccinated, each with two agar cultures of the strain "Pest Avirulent." Seventeen days later, upon testing the immunity of the animals, five died from the infection and five survived. Animal number 1379 evidently died of plague toxæmia and not of septicæmia.

In Series 51 (p. 203), fifteen monkeys were vaccinated subcutaneously with one or two 48-hour cultures of the strain "Pest Avirulent." The immunity of the animals was tested one month after the vaccination by the subcutaneous inoculation of two-thirds of an oese of the strain "Pest Virulent." Eight of the animals remained alive and seven died.

In all, forty-four monkeys were vaccinated with the strain "Pest Avirulent" and reinoculated with the strain "Pest Virulent;" 52 per cent of these animals proved to be immune.

Experiments with the strain "Pest Avirulent" in guinea pigs.—Seventy-one guinea pigs in Series 32, 37, 39, 41, and 46 p. 204 were inoculated, either intraperitoneally or subcutaneously, with from 1 to 2 agar cultures of the strain "Pest Avirulent." Such large doses as two 48-hour agar cultures, when injected intraperitoneally into the smaller guinea pigs (150 to 175 grams in weight), frequently gave rise to the death of the animals from toxæmia with, however, no evidence that a general invasion by the organisms had taken place. In only one instance (guinea pig number 1985, p. 205) two colonies of "Pest Avirulent" developed in cultures made from the heart at autopsy. Even one agar culture injected *intraperitoneally* caused the death of one small animal from pest intoxication, the animal dying in less than twenty-four hours after the vaccination. However, when the cultures were injected *subcutaneously,* even in small guinea pigs, death never occurred from the effect of the vaccination; this was demonstrated by the inoculation of fifty-one animals. (See Series 39, 41, and 46, pp. 208 to 212.) Five of the entire number of seventy-one guinea pigs vaccinated with this strain died from the intraperitoneal vaccination with 2 agar cultures, one from the intraperitoneal vaccination with 1 agar culture and one from an unknown cause. The remaining sixty-four were tested one and two months after the vaccination, whereupon forty-six (72 per cent) proved to be immune and twenty-five (38 per cent) died. The test of the immunity of the animals was severe and the skin was well scarified in each instance, two parallel incisions being made through the dermis with a scalpel and the suspension of the virulent organism rubbed into the incisions. Every one of 115 control animals, which were inoculated in exactly the same manner and at the same time as the vaccinated animals, died of pest infection. Guinea pig number 2087 was pregnant at the time of its vaccination and gave birth to two healthy young, seven days after its reinoculation.

Experiments in the immunization of monkeys with the strain "Maassen Alt."—Four monkeys in Series 17 (p. 212) were vaccinated, three with 2 agar cultures and one with 1 culture of the strain "Maassen Alt." Twenty days afterwards their immunity was tested by the inoculation of ½ oese of the strain "Pest Virulent," when all which had been vaccinated with the two cultures proved to be thoroughly immune, while the animal which had received only one culture died of pest. Every one of ten control (nonvaccinated) monkeys inoculated in the same manner died of the infection.

Twelve monkeys in Series 21 (p. 213) were vaccinated, each either with 1 or 2 cultures of this same strain, "Maassen Alt." On testing their immunity two weeks later by the inoculation of ½ oese of the strain "Pest Virulent" it was found that of the eight vaccinated with 2 cultures, two died after a somewhat prolonged infection and of the four vaccinated with 1 culture, two also died, one after a prolonged illness. Therefore, the mortality in this series was 33.3 per cent, 66.6 per cent of the monkeys having been immunized. Each of twelve control animals inoculated at the same time succumbed to pest infection.

Eighteen monkeys in Series 24 (p. 215) were inoculated with agar cultures of this organism ("Maassen Alt"), one with ½ a culture, and the remainder with 1 or 2 cultures. Four of these animals died after the vaccination. It seems clear that the animal numbered 1552 died from the effects of the vaccination and in the remaining three monkeys, death probably occurred both from the same cause and from that of infection with pyogenic cocci. Two weeks later on testing the immunity of the nine which were inoculated with 2 agar cultures and which survived the vaccination, all but one (number 1545) were found to resist the infection. This one died of pest. On testing the immunity of the four animals which had been inoculated with 1 culture and of the one which had been given ½ culture, all were found to be immune. Hence the entire mortality in this series was 28 per cent. However, as a number of the control animals inoculated at this time and in the same manner did not die, the remaining 72 per cent of the animals of this series can not be regarded as being of necessity highly immunized.

Fifteen other monkeys in Series 52 (p. 217) were inoculated subcutaneously each with 1 agar culture of the strain "Maassen Alt." One of the animals died thirteen days after vaccination. The cause of death could not be discovered at autopsy. One month after the vaccination, on testing the immunity of the remaining fourteen by the subcutaneous inoculation of ⅔ oese of the strain "Pest Virulent," seven died and seven survived the inoculation.

In all, forty-nine monkeys were vaccinated with the strain "Maassen Alt;" of these, four probably succumbed from the effects of the vaccination and one from an unknown cause. On testing the immunity of the remaining forty-four, thirteen died and thirty-one (70 per cent) were found to be immune.

Experiments in the immunization of guinea pigs with the strain "Maassen Alt."—Forty-seven guinea pigs in Series 33, 38, 40, and 47 (pp. 218 to 221) were inoculated either intraperitoneally or subcutaneously with 1 agar culture of the strain "Maassent Alt." In the experiments in which the inoculation was made intraperitoneally, a number of the animals died of pest intoxication, namely, three in Series 33 and one in Series 38. In three of these instances the cultures made at autopsy from the heart's blood of the animal remained sterile; in the fourth, no cultures were taken from the heart. Two of the animals died two days after the vaccination and, although in each instance cultures from the heart were sterile, in both cases cultures prepared from the abdominal cavity developed a rich growth of the strain "Maassen Alt." The guinea pigs also occasionally died from the subcutaneous inoculation of only 1 agar culture of the strain "Maassen Alt;" this was true of one in Series 40 and one in Series 47. In both instances the animals succumbed within twenty-four hours after the vaccination, evidently of plague toxæmia. Therefore, the strain "Maassen Alt" is distinctly more virulent and more toxic than "Pest Avirulent." To sum up, six in all of the forty-seven guinea pigs died from the effect of the vaccination. The immunity of the remaining forty-one was tested from one to two months after the vaccination, when only five (12 per cent) died and thirty-six (88 per cent) were shown to have been thoroughly immunized. One hundred and fifteen unvaccinated control guinea pigs were tested in exactly the same manner and at the same time as the vaccinated ones. All of the control animals died of pest infection.

3. IMMUNIZATION WITH FILTERED CULTURES AND EXTRACTS (FREE RECEPTORS) OF THE ORGANISM.

The German Plague Commission (Gaffky, Pfeiffer, Sticker, Dieudonné) reports two experiments in the immunization of *Macacus* monkeys with filtered bouillon cultures of the plague organism.

> A ten day's bouillon culture of the virulent bacillus was filtered through a Berkefeld filter, one portion of the filtrate was heated to 60° C. and another mixed with 0.5 per cent carbolic acid and put aside for twenty-four hours. One of the monkeys was inoculated with 5 cubic centimeters of the first portion and the other with 5 cubic centimeters of the second. On subsequently testing the immunity of these animals with 1 oese of the virulent, living pest bacillus, both succumbed to the infection.
>
> The Austrian Plague Commission (Albrecht and Gohn) found that a moderate degree of immunity could be obtained in rats with filtered bouillon cultures of the plague bacillus. The immunity which resulted from the use of the old bouillon cultures was higher than that which came from the young ones, but in neither instance did it equal that which was obtained with killed cultures of the organism.

Kossel and Overbeck[44] also stated that rats could sometimes be immunized with the filtrates of bouillon cultures which were killed by heating at from 56° to 60° C., although no details of the experiments are given. Markl[45] believed that a soluble pest toxin as well as some metabolic product was formed in the filtrate of young bouillon cultures and of those grown at a low temperature, with which antitoxic immunity could be obtained and an antitoxic serum produced in horses. The animals with which he experimented certainly acquired a tolerance against the injection of the filtered cultures, but the immunity against pest *infection* was not tested in any of them; moreover, Kolle[46] was unable to produce a serum of any value with such cultures, although from repeated inoculations of filtrates of bouillon cultures from eight to ten weeks old he was able to obtain a serum of very low agglutinative and protective power. It would appear from these experiments, that the plague bacillus does not readily undergo autolysis and liberate free antigenetic receptors even in old bouillon cultures.

Besredka[47] found that a separation of the pest endotoxin from the bacilli occurred when dried pest bacilli, physiologic salt solution and normal serum of the horse were mixed and allowed to stand over night at the temperature of the ice box.

Upon centrifuging this mixture and separating the bacteria, the clear fluid was found to contain most of the pest toxin. The precipitated bacteria were found to have lost their toxic action to a great extent, but not their immunizing power. Nine mice were each inoculated with 0.002 milligram of these bacilli "atoxique." Five of the animals survived the inoculation and the course of the infection was prolonged in the remaining four.

Owing to the success I had met with in immunizing both man and animals with the free receptors obtained from the cholera spirillum by autolysis, similar experiments were undertaken upon animals with the plague bacillus.

In the first experiments, 48-hour agar slant cultures of the virulent strain were suspended in a small quantity of distilled water, heated during one to two hours at 60° C., and after being placed for several days in the incubator at 37° C., were filtered through a Berkefeld candle.

Immunization of monkeys with the free receptors of the plague bacillus.—Experiments with such filtrates are recorded in Series 7 and 26 pp. 221 to 223. Nine monkeys were inoculated subcutaneously with various amounts of the free receptors. Only two were found to be immune and to survive the infection when their immunity was tested from ten to fourteen days after the first inoculation. The results of these experiments were so unfavorable that attempts to obtain the free receptors in a somewhat different manner were instituted.

The further experiments of this nature with the free receptors of the organism of pest will be described in the next section of this paper devoted to the subject of immunization with plague aggressin, since, owing to the work of Bail and his associates, the term "aggressin" has now become well established in medical literature.

[44] *Hyg. Rund.* (1901), **11**, 103.

[45] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1901), **37**, 401.

[46] Festschrift f. Robert Koch (1903), 352.

[47] *Ann. d. l'inst. Pasteur* (1905), **19**, 479.

4. IMMUNIZATION WITH PLAGUE AGGRESSIN.

Hueppe and Kikuchi were the first to perform experiments with natural plague aggressin in animals and to announce that a certain and not dangerous method had been discovered for immunization against pest. This work has already been referred to in the introduction to this article. Their experiments were few in number but the authors mention that it was their intention merely to call attention to the priority of the use of this method of immunization of animals against plague. The guinea pigs which were immunized had received several inoculations of the aggressin exudates.

(a) IMMUNIZATION WITH ARTIFICIAL AGGRESSIN.

Further attempts at immunization with the free receptors of the plague bacillus (artificial plague aggressin) were being performed in this laboratory at the time of the appearance of Hueppe and Kikuchi's work, but no very favorable results could be obtained. After the publication of their article the subject was investigated anew, although I had already made a preliminary report of the value of immunization with *artificial* plague aggressin on March 3, 1906.[48] Further experiments made in immunization with artificial plague aggressin are given in detail in Series 29, 30, 31, 36, and 42 (pp. 223 to 229), together with the method of preparation of the extract of the organism. In all, twenty-six guinea pigs and thirty-two monkeys were inoculated with the extracts of the strain "Pest Virulent." Only three of the guinea pigs (11 per cent) and but four of the monkeys (12.5 per cent) proved to be immune, when the immunity of the animals was tested from two to seven weeks after the first inoculation. These results were so unfavorable as compared with those in which the living, attenuated cultures of the pest organism were used for inoculation, that this method was not pursued further, but experiments with natural plague aggressin were undertaken on guinea pigs for the purpose also of comparing the immunizing power of this substance with that of the living attenuated organism.

(b) IMMUNIZATION IN GUINEA PIGS WITH EXUDATES FROM PLAGUE-INFECTED ANIMALS (NATURAL AGGRESSIN).

The protocols of the animals employed are given in Series 34 and 43 (p. 229), where the further details in the preparation of the aggressin exudates are also described. Fifteen of the guinea pigs of Series 35 (p. 230), were inoculated with the exudates obtained from the animals comprising Series 34, the guinea pigs each receiving, intraperitoneally from 2 to 5 cubic centimeters of the exudates. All survived the inoculation of the aggressin. Their immunity was tested with the virulent organism about two months after the first inoculation, when all but four (26+ per cent) succumbed to pest infection. Twenty-seven control animals were also inoculated at the same time in the same manner; these all died of pest. Twelve animals in Series 44 (p. 232) were inoculated with exudates obtained from the guinea pigs comprising Series 43. The immunity of the animals was tested about one month after the first inoculation; eight (66.6 per cent) died and four (33.3 per cent) were found to be immune.

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[48] *This Journal* (1906), 1, 501.

5. IMMUNIZATION OF ANIMALS BY KLEIN'S METHOD.

Only a few guinea pigs were inoculated with the prophylactic prepared after the method of Klein because it did not seem clear that it possessed any advantages over immunization with the aggressin exudates, while it appeared to cause more local irritation. Thirteen guinea pigs in Series 54 (p. 232) and 56 (p. 233) were each inoculated with a portion of the powder dissolved in 2 cubic centimeters of saline solution and obtained from dying at 46° to 47° C. and then triturating in a mortar the buboes, spleen, liver and lungs of guinea pigs which had died of subacute pest infection. Sloughs of the skin followed the inoculations in five instances. The immunity of the animals was tested with the virulent pest strain about one month after the first inoculation, when all but four (30 per cent) succumbed to pest infection.

Klein does not refer to a marked local reaction following the injection of his prophylactic, and perhaps such a reaction was in his experiments avoided by some details in the manufacture of the prophylactic not emphasized in his preliminary description of its preparation.

VI. SERIES OF ANIMAL INOCULATIONS EMPLOYED IN TESTING THE IMMUNIZING VALUE OF THE DIFFERENT METHODS.

EXPERIMENTS DEMONSTRATING SUSCEPTIBILITY OF MONKEYS TO PLAGUE INFECTION.

SERIES 1.—*With monkeys.*

One 48-hour agar slant culture of "Pest Virulent" was suspended in 5 cubic centimeters of bouillon. A 10 cubic centimeter syringe needle was dipped in such a suspension and the needle then thrust beneath the shaved skin of each animal in the region near the root of the tail, pushed in full length and then withdrawn.

Animal No.	Result.	Autopsy and remarks.
1222	Dead in 3 days	Pest septicæmia. *B. pestis* from heart, liver and spleen and hæmorrhagic inguinal glands.
1223	Dead in 4 days	Pest septicæmia. Innumerable pest bacilli in blood of heart.
2695	do	Pest septicæmia. *B. pestis* from heart, spleen and liver.
2696	Dead in 3½ days	Pest septicæmia. *B. pestis* from heart, spleen and liver.

SERIES 2.—*With monkeys.*

One 48-hour agar slant culture of "Pest Virulent" (second transplant from guinea pig number 1220) suspended in 5 cubic centimeters of bouillon. One cubic centimeter of the suspension equal to 2 oesen. Animals both inoculated subcutaneously near root of tail with 1 cubic centimeter.

Animal No.	Amount inoculated.	Result.	Autopsy and remarks.
1224	1 cc. (=2 oesen) subcutaneously.	Dead in 3 days.	Pest septicæmia. Smears from the heart, spleen, hæmorrhagic glands and liver show numerous pest bacilli.
1225	do	Dead in 2 days.	Pest septicæmia. Hæmorrhagic area about point of inoculation. Left inguinal glands swollen and hæmorrhagic. Spleen swollen. Innumerable pest bacilli from all organs.

SERIES 3.— *With monkeys and guinea pigs.*

One 48-hour agar slant culture of "Pest Virulent" suspended in 5 cubic centimeters of bouillon. A scalpel was dipped several times in such a suspension and then rubbed over a shaved area on the abdomen, which was also slightly scarified with the knife.

Animal No.	Result.	Autopsy and remarks.
Monkey 1226.	Dead in 5 days.	Found dead in the morning. On microscopical examination of stained-blood films from heart's blood, pest bacilli found present in very small numbers. Cultures from the heart developed colonies of the pest bacillus. A portion of the spleen of this animal was rubbed over the shaved abdomen of monkey No. 1244 (see below).
Monkey 1227.	Dead in 4 days.	Plague septicæmia. Hæmorrhagic glands. Cover slip specimens from heart's blood reveal innumerable pest bacilli.
Monkey 1286.	Alive	
Monkey 1287.	do	

The abdomen of each animal was shaved over a small area and scarified lightly, then a portion of the spleen of another animal which had succumbed to acute pest infection was rubbed over this area.

Animal No.	Abdomen massaged with spleen of—	Result.	Autopsy and remarks.
Monkey 1244	Monkey No. 1226 (see above).	Alive and well.	
Guinea pig 1170.	Monkey No. 1288 (see Series 5, Oct. 25).	Dead Oct. 30, after 5 days.	Buboes and multiple necrotic foci in spleen and liver. Pure cultures of *Bacillus pestis* from the heart.
Guinea pig 1296.	do	do	Buboes and multiple necrotic foci in spleen and liver. Numerous pest bacilli in smears from spleen.

SERIES 16.— *With monkeys.*

On November 2, two 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1296, second transplant) were suspended in 10 cubic centimeters of bouillon (5 cubic centimeters to each tube), a 10 cubic centimeter syringe needle was dipped in this suspension and thrust beneath the skin of monkeys numbers 1357 to 1360 inclusive. Monkeys numbers 1361 to 1364 in-

SERIES 16—Continued.

clusive each received subcutaneously 0.5 cubic centimeter of this same suspension (equal to 1 oese "Pest Virulent") and monkeys numbered 1365 to 1368 each received subcutaneously 0.25 cubic centimeters, equal to ½ oese.

Animal No.	How inoculated.	Result.	Remarks and autopsy.
1357	Stuck with 10 cc. needle dipped in suspension "Pest Virulent."	Alive and well	Animal suffered a mild infection and became immune, resisting the reinoculation of 2 whole oesen "Pest Virulent" on Dec. 14.
1358	do	do	Do.
1359	do	Dead Nov. 7, after 5 days.	Innumerable pest bacilli in smears from the spleen.
1360	do	Dead Nov. 9, after 7 days.	Innumerable pest bacilli in smears from the spleen. Pest septicæmia.
1361	1 oese "Pest Virulent" subcutaneously.	Dead Nov. 6, after 4 days.	Innumerable pest bacilli in smears from the spleen.
1362	do	Dead Nov. 5, after 3 days.	Do.
1363	do	do	Do.
1364	do	do	Do.
1365	½ oese "Pest Virulent" subcutaneously.	Dead Nov. 7, after 5 days.	Do.
1366	do	do	Do.
1367	do	do	Do.
1368	do	Dead Nov. 6, after 4 days.	Do.

EXPERIMENTS IN IMMUNIZATION OF ANIMALS WITH KILLED CULTURES OF THE PEST BACILLUS.

SERIES 5.—*Killed bouillon cultures, with monkeys.*

The following monkeys were inoculated subcutaneously near the root of the tail with Haffkine's plague prophylactic purchased by the Bureau of Health from India. The immunity of all the animals was tested eleven days after the first injection, in the following manner: One 24-hour agar slant culture of "Pest Virulent" was suspended in 5 cubic centimeters of saline solution; a 10 cubic centimeter syringe needle was dipped in this suspension and then thrust beneath the shaved skin of the animal on the opposite side of the tail to which the first injection had been made.

SERIES 5—Continued.

Animal No.	Amount of prophylactic inoculated.	Result after infection.	Autopsy and remarks.
1234	5 cc	Dead after 5 days	Pest septicæmia. Innumerable pest bacilli in heart's blood.
1235	5 cc	do	Do.
1236	10 cc	do	Numerous pest bacilli in smears from spleen.
1237	30 cc	Dead after 7 days	Extensive hæmorrhages in the mesentery. Numerous pest bacilli in smears from spleen.
1238	20 cc	Dead after 14 days	Only one or two bacilli found in smears from spleen. Innumerable in smears from large necrotic bubo near region of left buttock (point of inoculation)—chronic pest.
1239	20 cc	Alive	
1240	10 cc	do	
1241	30 cc	do	
1288	Control	Dead after 3½ days	Numerous pest bacilli in smears from the blood and spleen.
1289	Control	Dead after 7 days	Numerous pest bacilli in smears from spleen.

SERIES 9.—*Killed agar cultures "Pest Virulent," with monkeys.*

Six 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1221, second transplant) suspended in 6 cubic centimeters of saline solution (1 cubic centimeter to each tube), the whole mixed and heated for one and one-half hours at 62° C. A culture taken proved the suspension to be sterile. The following animals were inoculated subcutaneously, each with 2 cubic centimeters (equal to 2 agar slant cultures) on October 18. On October 28 the animals were reinoculated subcutaneously on the other side of the body with 1 cubic centimeter of a suspension of a 48-hour slant culture of living "Pest Virulent" in 5 cubic centimeters of bouillon (1 cubic centimeter equal to 2 oesen).

Animal No.	Immunized October 18.	Infected October 28.	Result.	Autopsy and remarks.
1261	2 agar slant cultures.	2 oesen "Pest Virulent" subcutaneously.	Dead after 4 days.	Pest septicæmia.
1262	do	do	Dead after 3 days.	Numerous pest bacilli in smears from spleen.
1263	do	do	do	Innumerable pest bacilli in smears from spleen.
		CONTROL.		
1322		2 oesen "Pest Virulent" subcutaneously.	Dead after 3 days.	Innumerable pest bacilli in spleen.

SERIES 23.—*Killed and living agar cultures of "Pest Virulent" with monkeys.*

Twenty-five 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1496, second transplant) were suspended in 25 cubic centimeters of bouillon. The suspension was placed in an incubator registering 60° C. for one and one-half hours. After its removal, cultures were taken, which, two days later, developed colonies of pest bacilli (three colonies to two large loops of the suspension.) Evidently, the temperature of the very concentrated suspension of bacilli had not reached 60° C., although a thermometer placed in a test tube containing bouillon in the incubator registered this temperature during the heating. With this suspension containing killed and also a moderate number of living pest bacilli this series of animals was inoculated as below on December 4, 1906. On December 18, two weeks after the first inoculation, the animals which remained alive were reinoculated in the following manner. Nine 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1516, second transplant) were suspended in 45 cubic centimeters of bouillon, and each animal was reinoculated subcutaneously on the opposite side of the body to that on which the first inoculation was made with 0.2 cubic centimeter of this suspension, that is with a little less than ½ oese of "Pest Virulent."

Animal No.	Inoculated December 4 subcutaneously with—	Infected December 18 with—	Result.	Autopsy and remarks.
1518	1 killed agar slant "Pest Virulent."		Dead from first inoculation Dec. 6, after 2 days.	A few pest bacilli in smears from the spleen.
1519	do		do	Very numerous pest bacilli in smears from spleen.
1520	do	Nearly ½ oese "Pest Virulent."	Dead Dec. 20	Culture from the spleen negative. Numerous pest bacilli in smears from the bubo.*
1521	do	do	Alive	
1522	do	do	do	
1523	do	do	do	
1524	do	do	do	
1525	do	do	do	
1526	do	do	do	
1527	do		Dead from first inoculation Dec. 6, after 2 days.	Smears from point of inoculation show no bacilli. Cultures from spleen show numerous pest bacilli.
1528	2 killed agar slants "Pest Virulent."	Nearly ½ oese "Pest Virulent."	Dead Jan. 4, after 17 days.	Numerous pest bacilli in smears from spleen. Large suppurating bubo.
1529	do	do	Alive	
1530	do	do	do	
1531	do	do	do	
1532	do	do	do	
1533	do	do	Dead Dec. 24, after 6 days.	Abscess at point of inoculation containing pest bacilli.
1534	do	do	Alive	
1535	⅘ killed agar slant "Pest Virulent."	do	Dead Dec. 20, after 2 days.	Very numerous pest bacilli in smears from spleen.*

*It is possible that these animals were suffering with a latent or chronic form of pest at the time of the second reinoculation, although they were apparently healthy.

SERIES 23—Continued.

CONTROLS.				
Animal No.	Inoculated December 4 subcutaneously with—	Infected December 18 with—	Result.	Autopsy and remarks.
1594	----------	Nearly ½ oese "Pest Virulent."	Alive and well	
1595	----------	do	do	
1596	----------	do	do	
1597	----------	do	Dead Dec. 25, after 7 days.	Pest bubo with innumerable bacilli.
1598	----------	do	do	Do.
1599	----------	do	do	Do.
1600	----------	do	do	Pest bubo with innumerable bacilli. Innumerable bacilli in the spleen.
1601	----------	do	Dead Dec. 26, after 8 days.	Do.
1602	----------	do	Alive and well	Do.
1603	----------	do	Dead Jan. 3	No evidence of pest infection.
1604	----------	do	Dead Dec. 27, after 9 days.	Pest bubo containing numerous pest bacilli. No organisms seen in a smear from spleen.
1605	----------	do	Alive and well	

*It is possible that these animals were suffering with a latent or chronic form of pest at the time of the second reinoculation, although they were apparently healthy.

The majority of the monkeys of this series, including the controls, were very large, weighing 5,000 grams and over, the large animals having been collected and saved for inoculation in the same series. A number of the controls, as will be seen from an examination of the table, did not die, and hence the exact value of the immunization is not shown by the experiments. In the series of inoculations performed after this date, whenever the monkey's weight was over 3,000 grams, ⅔ oese of "Pest Virulent" was employed in testing the immunity, in place of ½ oese. However, some of the very large monkeys were apparently as susceptible to pest infection as certain of the small ones. The series on the other hand shows very distinctly an important fact, namely, that it is not possible to immunize all animals of this species with a single uniform dose, for, although the primary inoculation was so large that it killed some of the animals of this series, in other instances it failed to protect them against the subsequent inoculation of even less than ½ oese of "Pest Virulent," which amount, in even some of the control animals, did not give rise to a fatal infection. The animals that succumbed to the first inoculation also demonstrate the inefficacy of a single heating in attenuating the pest bacillus, the few bacilli remaining alive in the suspension having retained their full virulence.

SERIES 25.—*Killed agar cultures of "Pest Virulent" with monkeys.*

On December 8, twenty-eight 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1514, second transplant) were suspended in 28 cubic centimeters of 0.085 saline solution (1 cubic centimeter to each culture) and the suspension placed at 65° C. for one hour. Cultures from this suspension afterwards proved to be sterile. Monkeys numbered 1558 to 1571 inclusive were

SERIES 25—Continued.

inoculated subcutaneously each with 1 cubic centimeter, monkeys numbered 1572 to 1576 each with 2 cubic centimeters and number 1577 with 0.8 cubic centimeter. Three of the animals apparently succumbed from the effect of this inoculation. On December 26, eighteen days after the first inoculation, the animals were reinoculated in the following manner: Six 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1592, second transplant) were suspended in 30 cubic centimeters bouillon (5 cubic centimeters to each tube). Each animal was inoculated subcutaneously on the opposite side of the body to that on which the first inoculation was made with 0.33 cubic centimeter, equal to ⅔ oese "Pest Virulent." Twelve control animals were also inoculated at the same time with the same amount of the suspension.

Animal No.	Inoculated December 8 subcutaneously with—	Reinoculated December 26 with—	Result.	Autopsy and remarks.
1558	1 killed culture "Pest Virulent."	⅔ oese "Pest Virulent."	Dead Dec. 28, after 2 days.	Innumerable pest bacilli in smears from spleen.
1559	do	do	Dead Jan. 10, after 15 days.	Chronic pest. Very numerous pest bacilli in smears from spleen.
1560	do	do	Alive	
1561	do	do	Dead Dec. 28, after 2 days.	Fair number of pest bacilli in smears from the spleen. Very numerous about point of inoculation. Tissues œdematous and hæmorrhagic.
1562	do	do	Dead Dec, 28, after 2½ days.	Numerous pest bacilli in smears from the bubo. Culture from spleen negative.
1563	do	do	Dead Jan. 5, after 10 days.	Numerous pest bacilli in smears from spleen. Large bubo.
1564	do	do	Alive and well	
1565	do		Died of first inoculation.	Nothing to suggest pest about point of inoculation or in the spleen. Smears from spleen negative.
1566	do	⅔ oese "Pest Virulent."	Dead Jan. 4, after 9 days.	Numerous pest bacilli in smears from spleen. Bubo.
1567	do	do	Dead Dec. 30, after 4 days.	Few organisms in smears from the spleen. Innumerable about point of inoculation. Intense hæmorrhagic reaction.
1568	do	do	Dead Dec. 27, after 1½ days.	Innumerable pest bacilli from point of inoculation.
1569	do	do	Dead Jan. 2, after 7 days.	Few bacilli in smears from spleen. Innumerable from bubo.
1570	do		Dead Dec. 11, from primary inoculation.	Nothing to suggest pest except small hæmorrhages in the lungs. Nothing suggestive at the point of inoculation. Cultures from the spleen negative although large amount is inoculated.
1571	do	⅔ oese "Pest Virulent."	Alive and well	

Series 25—Continued.

Animal No.	Inoculated December 8 subcutaneously with—	Reinoculated December 26 with—	Result.	Autopsy and remarks.
1572	2 killed cultures "Pest Virulent."		Dead Dec. 24, from primary inoculation.	No evidences of pest. Animal emaciated. Tissues softened, hæmorrhagic and necrotic near point of infection.
1573	do	⅔ oese "Pest Virulent."	Dead Jan. 6, after 11 days.	Numerous pest bacilli in smears from spleen. Large suppurating bubo.
1574	do	do	Dead Jan. 7, after 12 days.	Numerous pest bacilli in smears from spleen. Large bubo.
1575	do	do	Dead Dec. 31, after 5 days.	Fair numbers of pest bacilli in smears from the spleen.
1576	do	do	Alive and well	
1577	⅘ killed culture "Pest Virulent."	do	Dead Jan. 6, after 11 days.	Fair number of pest bacilli in smears from the spleen. Large suppurating bubo.
CONTROLS.				
1613		⅔ oese "Pest Virulent."	Dead Dec. 30, after 4 days.	Innumerable pest bacilli in smears from spleen.
1614		do	Dead Oct. 29, after 3 days.	Do.
1615		do	Dead Dec. 30, after 4 days.	Fair numbers of pest bacilli in smears from the spleen.
1616		do	Dead Dec. 30, after 4 days.	Innumerable pest bacilli in smears from the spleen.
1617		do	Dead Dec. 29, after 3 days.	Do.
1618		do	Dead Jan. 2, after 7 days.	Very numerous pest bacilli in smears from spleen. Also many post-mortem bacilli.
1619		do	Dead Dec. 31, after 5 days.	Innumerable pest bacilli in smears from spleen.
1620		do	Dead Dec. 28, after 2 days.	Few pest bacilli in smears from spleen. Innumerable in smears near point of inoculation. No pus formation at this point. The tissues hæmorrhagic.
1621		do	do	Considerable number pest bacilli in smears from spleen. Tissues œdematous and hæmorrhagic near point of inoculation in which pest bacilli are very numerous.
1622		do	Dead Jan. 2, after 7 days.	Few pest bacilli in smears from spleen.
1623		do	Dead Jan. 12, after 17 days.	Numerous pest bacilli in smears from spleen. Large open bubo filled with pus.
1624		do	Dead Jan. 4, after 9 days.	Fair numbers of pest bacilli in smears from the spleen. Bubo.

SERIES 48.—*Killed agar cultures of "Pest Virulent" with monkeys.*

Fifteen 48-hour agar cultures of the strain "Pest Virulent" from guinea pig number 2583, second transplant, were suspended in 15 cubic centimeters of saline solution (1 cubic centimeter to each culture), the entire suspension heated for one hour and fifteen minutes at 65° C. Cultures taken from the suspension remained sterile.

On October 27 each monkey of the series was inoculated subcutaneously with 1 cubic centimeter of the suspension (equal to one agar slant culture). On November 28, one month after the inoculations, the immunity of the animals was tested by the subcutaneous injection of 0.33 cubic centimeter of a suspension of the strain "Pest Virulent" (five 48-hour agar cultures from guinea pig number 2865, second transplant in 25 cubic centimeters bouillon) into the opposite side of the body to that on which the vaccination was made. Twenty-five control animals were also inoculated on this date and in the same manner. The immunity of the animals of this series was tested on the same date and in the same manner as was that of the animals comprising Series 49, 51, and 52 (pp. 197, 203, 217).

Animal No.	Vaccinated October 27 subcutaneously with—	Reinoculated November 28 with—	Result.	Autopsy and remarks.
2664	One killed 48-hour culture "Pest Virulent."	⅔ oese "Pest Virulent."	Dead Dec. 2, after 4 days.	Few pest bacilli in smears from spleen. Numerous at point of inoculation. Buboes.
2665	do	do	Alive and well.	
2666	do	do	do	
2667	do	do	Dead Dec. 1, after 3 days.	Innumerable pest bacilli in smears from spleen.
2668	do	do	do	Innumerable pest bacilli in smears from spleen.
2669	do	do	Dead Dec. 2, after 4 days.	Few pest bacilli in smears from spleen.
2670	do	Not reinoculated.	Dead Nov. 5, of first inoculation.	Careful post-mortem does not disclose cause of death. No marked local reaction.
2671	do	⅔ oese "Pest Virulent."	Dead Dec. 3, after 5 days.	Buboes and pest bacilli.
2672	do	do	Dead Dec. 1, after 3 days.	Do.
2673	do	do	Dead Dec. 3, after 5 days	Do.
2674	do	do	Dead Dec. 1, after 3 days.	Do.
2675	do	do	Dead Dec. 2, after 4 days.	Innumerable pest bacilli in smears from spleen.
2676	do	do	Alive and well.	
2677	do	do	do	
2678	do	do	Dead Dec. 1, after 3 days.	Fair number of pest bacilli in smears from spleen.

SERIES 48—Continued.

CONTROLS.

Animal No.	Vaccinated October 27 subcutaneously with—	Reinoculated November 28 with—	Result.	Autopsy and remarks.
2896		$\frac{2}{3}$ oese "Pest Virulent."	Dead Dec. 2, after 4 days.	Innumerable pest bacilli in smears from spleen.
2897		do	Dead Dec. 3, after 5 days.	Do.
2898		do	Dead Dec. 1, after 3 days.	Do.
2899		do	Dead Dec. 2, after 4 days.	Do.
2900		do	do	Do.
2901		do	do	Do.
2902		do	Dead Dec. 1, after 3 days.	Do.
2903		do	do	Do.
2904		do	do	Do.
2905		do	Dead Dec. 2, after 4 days.	Do.
2906		do	Dead Dec. 1, after 3 days.	Do.
2907		do	Dead Dec. 2, after 4 days.	Do.
2908		do	do	Do.
2909		do	Dead Dec. 3, after 5 days.	Do.
2910		do	Dead Dec. 1, after 3 days.	Do.
2911		do	Dead Dec. 3, after 5 days.	Do.
2912		do	do	Do.
2913		do	Dead Dec. 2, after 4 days.	Do.
2914		do	Dead Dec. 1, after 3 days.	Do.
2915		do	Dead Dec. 2, after 4 days.	Do.
2916		do	do	Do.
2917		do	Dead Dec. 1, after 3 days.	Do.
2918		do	Dead Dec. 3, after 5 days.	Do.
2919		do	Dead Dec. 4, after 6 days.	Do.
2920		do	Dead Dec. 1, after 3 days.	Do.

SERIES 49.—*Killed bouillon cultures of "Pest Virulent," with monkeys.*

The monkeys of this series were inoculated on October 27, subcutaneously near the root of the tail with from 10 to 15 cubic centimeters of six weeks' old bouillon cultures of the virulent pest organism killed by heating for one hour at 65° C. On November 28, one month after the primary inoculation, the immunity of the animals was tested by the subcutaneous injection of ⅔ oese of the strain "Pest Virulent" (from a 48-hour agar culture from guinea pig number 2865, second transplant), suspended in 0.33 cubic centimeter saline solution. The animals were inoculated on the opposite side of the body from that upon which the primary inoculation was made. Twenty-five control animals, numbered 2896 to 2920, were also inoculated on this date and in the same manner. They all died of pest infection. For the details of the autopsies see Series 48. The immunity of the animals of this series was tested on the same date and in the same manner as was that of the animals comprising Series 48, 51 and 52, pp. 195, 203, 217.

Animal No.	Inoculated October 27 subcutaneously with—	Reinoculated November 28 with—	Result.	Autopsy and remarks.
2697	15 cc. killed bouillon culture.	⅔ oese "Pest Virulent."	Dead Dec. 7, after 9 days.	Bubo containing pest bacilli.
2698	do	do	Dead Dec. 5, after 7 days.	Buboes containing pest bacilli.
2699	do	do	Alive and well	
2700	do	do	Dead Dec. 5, after 7 days.	Do.
2701	do	do	Dead Dec. 8, after 10 days.	Do.
2702	10 cc. killed bouillon culture.	do	Dead Dec. 3, after 5 days.	Do.
2703	do	do	Alive and well	
2704	do	do	Dead Dec. 1, after 3 days.	Do.
2705	do	do	Dead Dec. 2, after 4 days.	Do.

SERIES 50.—*Killed agar culture of "Pest Virulent," with guinea pigs.*

Fifteen 48-hour agar cultures of the strain "Pest Virulent" from guinea pig number 2583, second transplant, were suspended in 15 cubic centimeters of saline solution (1 cubic centimeter to each culture). The entire suspension was heated for one hour and fifteen minutes at 65° C. Cultures taken from the suspension remained sterile. On October 27 each guinea pig of the series was inoculated

SERIES 50—Continued.

subcutaneously with 1 cubic centimeter of the suspension, equal to 1 agar slant culture. On November 26, one month after vaccination, the immunity of the animals in each instance was tested with the strain "Pest Virulent" in exactly the same manner as in Series 46, 47, and 54 (pp. 211, 220, 232). Twenty-five control animals, numbered 2870 to 2894, were inoculated at the same time and in the same manner, all of which died of acute plague infection. For the details of the protocols see Series 46 (p. 211).

Animal No.	Vaccinated October 27 subcutaneously with—	Reinoculated November 26 with—	Result.	Autopsy and remarks.
2679	One 48-hour culture killed "Pest Virulent."	5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Alive and well	
2680	do	do	do	
2681	do	do	do	
2682	do	do	Dead Dec. 4, after 8 days.	Numerous pest bacilli in smears from spleen.
2683	do	do	Dead Dec. 12, after 16 days.	Large bubo. Typical pest spleen.
2684	do	do	Dead Dec. 5, after 9 days.	Typical pest bubo and spleen.
2685	do	do	Dead Dec. 10, after 14 days.	Do.
2686	do	do	Dead Dec. 13, after 17 days.	Multiple buboes. Infarcts in lung and spleen.
2687	do	do	Dead Dec. 10, after 14 days.	Typical bubo and spleen.
2688	do	do	Dead Dec. 4, after 8 days.	Do.
2689	do	do	Dead Dec. 10, after 14 days.	Do.
2690	do	do	Alive and well	
2691	do	do	Dead Dec. 8, after 12 days.	Do.
2692	do	do	Dead Dec. 4, after 8 days.	Do.
2693	do	do	Dead Dec. 7, after 11 days.	Do.

EXPERIMENTS IN THE IMMUNIZATION OF ANIMALS WITH LIVING, ATTENUATED CULTURES (VACCINATION).

SERIES 4.—*Inoculations with "Pest Avirulent" in monkeys.*

Six 24-hour agar slant cultures of "Pest Avirulent" were suspended in 6 cubic centimeters of bouillon (1 cubic centimeter to each culture), the whole mixed and 1 cubic centimeter of the mixed suspension injected subcutaneously near the root of the tail of each animal on October 10. Ten days later the immunity of the animals was tested in the following manner: A 48-hour agar culture of "Pest Virulent" (from guinea pig number 1221, second transplant) was suspended in 5 cubic centimeters of bouillon and animals numbered 1228 to 1231 and controls numbered 1282 to 1285 were reinoculated by dipping a 10 cubic centimeter syringe needle in this suspension and then thrusting the needle beneath the shaven skin near the root of the tail, on the opposite side to that upon which the vaccination had been made. Animals numbered 1232 and 1233 and controls numbered 1286 and 1287 were reinoculated by dipping a scalpel several times in this same suspension and rubbing it over the shaved abdomen and slightly scarifying the skin with the knife.

Animal No.	Vaccination October 10.	Reinoculated October 20.	Result.	Autopsy and remarks.
1228	1 agar culture.	By sticking with infected needle.	Dead after 8 days.	Numerous pest bacilli from the spleen.
1229	do	do	Alive	
1230	do	do	do	
1231	do	do	do	
1232	do	By scarification with infected scalpel.	do	
1233	do	do	do	
		CONTROLS.		
1282		By sticking with infected needle.	Dead after 6 days.	Innumerable pest bacilli in smears from heart's blood and spleen.
1283		do	Dead after 8 days.	Numbers of pest bacilli in smears from the spleen.
1284		do	Dead after 10 days.	A few pest bacilli in smears from the spleen. Very numerous in smears from the swollen hæmorrhagic glands.
1285		do	Alive	
1286		By scarification with infected scalpel.	do	
1287		do	do	

SERIES 11.—*Inoculations with "Pest Avirulent" in monkeys.*

Ten 24-hour agar slant cultures (—1 per cent alkaline to phenol phthaleïn)* of the strain "Pest Avirulent" were suspended each in 1 cubic centimeter of saline solution, the whole 10 cubic centimeters mixed and 1 cubic centimeter, or about 1 agar slant, injected into each animal subcutaneously near the root of the tail. Eleven days later the immunity of the animals was tested in the following manner: Two 48-hour agar cultures of "Pest Virulent" (monkey number 1295, second transplant) were suspended each in 5 cubic centimeters of bouillon, and each animal was reinoculated subcutaneously on the opposite side of the body with 1 cubic centimeter of this suspension, equal to 2 oesen.

Animal No.	Vaccination October 19.	Reinoculated October 30.	Result.	Autopsy and remarks.
1277	1 agar culture.	2 oesen "Pest Virulent."	Dead Nov. 6 (7 days).	Smears from the spleen revealed only a few swollen bacilli. In smears from the heart no organisms were found. In smears from the tissues at the point of inoculation the organisms are numerous. Cultures from the heart's blood (2 cultures) negative; plenty of blood inoculated.†
1278	do	do	Alive and well.	
1279	do	do	Dead Nov. 2 (3 days).	Innumerable pest bacilli in smears from the spleen.
1280	do	do	Dead Nov. 6 (7 days).	Smears from the spleen show only a few degenerating bacilli. Those from the heart show no organisms. Those from the bubo near point of inoculation show numerous pest bacilli. Cultures from the heart reveal 6 colonies only of pest bacilli.†
1281	do	do	Dead Nov. 7 (8 days).	Very numerous pest bacilli in smears from the spleen.
		CONTROLS.		
1325	None	2 oesen "Pest Virulent."	Dead Nov. 2 (3 days).	Innumerable pest bacilli in smears from the spleen.
1326	do	do	Dead Nov. 1 (2 days).	Innumerable pest bacilli in smears from the spleen. Culture from the heart=rich growth.
1327	do	do	Dead Nov. 2 (3 days).	Innumerable pest bacilli in smears from spleen.
1328	do	do	do	Do.
1329	do	do	Dead Nov. 3 (4 days).	Do.

* The growth of the pest bacilli on this agar appeared lighter than it did on +1 per cent acid agar which was used in all the other experiments.

† The blood of these animals has apparently acquired considerable anti-infectious power, if one may judge from the distribution of the bacilli.

SERIES 12.—*Inoculations with "Pest Avirulent" in monkeys.*

Thirteen 24-hour agar slant cultures of "Pest Avirulent" were suspended each in 1 cubic centimeter of bouillon, the suspensions were mixed and monkeys numbered 1298 to 1301 inclusive were each inoculated subcutaneously with 2 cubic centimeters and monkeys numbered 1302 to 1306 inclusive each with 1 cubic centimeter. Ten days after the vaccination the immunity of each animal was tested in the following manner: Two agar slant 48-hour cultures of "Pest Virulent" (from guinea pig number 1324, second transplant) were each suspended in 5 cubic centimeters of bouillon and animals numbered 1298 and 1300 to 1305, and controls numbered 1414 to 1422 were each inoculated subcutaneously with 0.25 cubic centimeter of this suspension, equal to ½ oese "Pest Virulent;" and animal number 1306, with 0.5 cubic centimeter, equal to 1 oese.

Animal No.	Vaccination October 25.	Reinoculated November 10.	Result.	Autopsy and remarks.
1298	2 agar slants "Pest Avirulent."	½ oese "Pest Virulent."	Dead Nov. 22 (12 days).	No pest bacilli in smears from spleen. Large suppurating bubo about point of inoculation containing pest bacilli and many cocci and other bacilli.
1299	-----do--------	----------------	----------------	Found dead about 12 hours after vaccination. There is a large suppurating wound over the abdomen. Cultures from this area developed colonies which were later identified as those of staphylococci and streptococci and of a colon bacillus. Cultures from the spleen show many staphylococci. Cultures near the point of inoculation show a few colonies of "Pest Avirulent" which have evidently survived.
1300	-----do--------	½ oese "Pest Virulent."	Alive and well.	
1301	-----do--------	-----do--------	-----do-------	
1302	1 agar slant "Pest Avirulent."	-----do--------	-----do-------	
1303	-----do--------	-----do--------	Dead Nov. 20 (10 days).	No bacilli found in smears from spleen. Suppurating bubo about point of inoculation containing pest bacilli.
1304	-----do--------	-----do--------	Dead Nov. 16 (6 days).	Very numerous pest bacilli in smears from spleen.
1305	-----do--------	-----do--------	Dead Nov. 17 (7 days).	No organisms found in smears from spleen. Cultures from the heart, negative. Bubo at point of inoculation comparatively small. A number of pest bacilli present.
1306	-----do--------	1 oese "Pest Virulent."	Alive and well.	

SERIES 12—Continued.

CONTROLS.

Anmial No.	Vaccination October 25.	Reinoculated November 10.	Result.	Autopsy and remarks.
1414	None	½ oese "Pest Virulent."	Dead Nov. 20 (10 days).	Few pest bacilli in smears from spleen. Chronic bubo.
1415	do	do	Dead Nov. 13 (3 days).	Innumerable pest bacilli in smears from spleen.
1416	do	do	Dead Nov. 14 (4 days).	Countless pest bacilli in smears from spleen.
1417	do	do	do	Do.
1418	do	do	Dead Nov. 15 (5 days).	Do.
1419	do	do	Dead Nov. 14 (4 days).	Do.
1420	do	do	Dead Nov. 13 (3 days).	Only comparatively few pest bacilli in smears from spleen. Œdematous infiltration and countless numbers near point of inoculation.
1421	do	do	do	Very numerous pest bacilli in smears from spleen.
1422	do	do	Dead Nov. 14.	Countless pest bacilli in smears from spleen. Hæmorrhagic infarction of the intestine.

SERIES 18.—*Inoculations with "Pest Avirulent" in monkeys.*

On November 6, twenty 48-hour agar cultures of "Pest Avirulent" were suspended in 20 cubic centimeters of bouillon and each animal was vaccinated with 2 cubic centimeters of this suspension, equal to 2 agar slant cultures. On November 23, seventeen days after the first inoculation, two 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1452, second transplant) were suspended in 10 cubic centimeters of bouillon and each animal was reinoculated subcutaneously, on the opposite side of the body to that upon which the first inoculation was performed, with 0.25 cubic centimeter of this suspension, equal to ½ oese. Ten control animals were also inoculated at the same time with the same dose.

Animal No.	Vaccinated November 6 subcutaneously with—	Reinoculated November 23 with—	Result.	Autopsy and remarks.
1373	2 agar cultures "Pest Avirulent."	½ oese "Pest Virulent."	Dead Nov. 29, after 6 days.	A few typical pest bacilli in smears from spleen.
1374	do	do	Alive and well	
1375	do	do	do	
1376	do	do	do	
1377	do	do	Dead Nov. 29, after 6 days.	Pest bacilli very numerous in smears from spleen.
1378	do	do	Alive and well	

SERIES 18—Continued.

Animal No.	Vaccinated November 6 subcutaneously with—	Reinoculated November 23 with—	Result.	Autopsy and remarks.
1379	2 agar cultures "Pest Avirulent."	½ oese "Pest Virulent."	Dead Dec. 6, after 13 days.	A large sloughing ulcer 4 centimeters in diameter about point of inoculation. No plague bacilli in cultures and smears from the spleen. Many bacilli and cocci in smears from the ulcer. The animal has evidently died of plague intoxication, the organisms multiplying locally.
1380	do	do	Dead Nov. 30, after 7 days.	A fair number of pest bacilli in smears from spleen.
1381	do	do	Dead Dec. 2, after 9 days.	Only two involution forms of organism found in smears from spleen; there is a bubo about the point of inoculation in smears from which a number of pest bacilli are present.
1382	do	do	Alive and well	

CONTROLS NUMBERS 1484 TO 1494.

These controls constitute the same animals given in Series 17. They all died of acute pest infection in from four to five days. For details of the autopsies see Series 17 (p. 213).

SERIES 51.—*Inoculations with "Pest Avirulent" in monkeys.*

Twenty-five 48-hour agar slant cultures of the strain "Pest Avirulent" were suspended in 25 cubic centimeters of saline solution (1 cubic centimeter to each culture). Monkeys numbered 2706 to 2712 each received subcutaneously 2 cubic centimeters of this suspension, equal to 2 agar cultures, and monkeys numbered 2713 to 2720 each received 1 cubic centimeter equal to 1 agar culture. On November 28, one month after the vaccination, the immunity of the animals was tested with a suspension of the strain "Pest Virulent" in exactly the same manner as was the immunity of the animals comprising Series 48 and 52 (p. 217). Twenty-five control animals, numbered 2896 to 2920, were also inoculated on this date and with the same suspension. They all died of acute pest infection. For the details of the protocols see Series 48 (p. 195).

Animal No.	Vaccinated October 29 subcutaneously with—	Reinoculated November 28 subcutaneously with—	Result.	Autopsy and remarks.
2706	Two 48-hour agar cultures "Pest Avirulent."	⅔ oese "Pest Virulent."	Dead Dec. 1, after 3 days.	Few pest bacilli in smears from spleen. Numerous at point of inoculation.
2707	do	do	Alive and well	
2708	do	do	do	
2709	do	do	Dead Dec. 4, after 6 days	Buboes with few pest bacilli.
2710	do	do	Alive and well	
2711	do	do	do	
2712	do	do	do	

SERIES 51—Continued.

Animal No.	Vaccinated October 29 subcutaneously with—	Renoculated November 28 subcutaneously with—	Result.	Autopsy and remarks.
2713	One 48-hour agar culture "Pest Avirulent."	$\frac{2}{5}$ oese "Pest Virulent."	Dead Dec. 1, after 3 days.	Numerous pest bacilli in smears from spleen.
2714	do	do	Dead Dec. 2, after 4 days.	Do.
2715	do	do	Dead Dec. 3, after 5 days.	Bubo and numerous pest bacilli in smears from spleen.
2716	do	do	Dead Dec. 1, after 3 days.	Numerous pest bacilli in smears from spleen.
2717	do	do	Alive and well	
2718	do	do	do	
2719	do	do	Dead Dec. 1, after 3 days.	Numerous pest bacilli in smears from spleen.
2720	do	do	Alive and well	

SERIES 32.—*Inoculations with "Pest Avirulent" in guinea pigs.*

May 16: Twenty-four 48-hour agar slant cultures of "Pest Avirulent" were suspended in 24 cubic centimeters of saline solution (1 cubic centimeter to each culture). Animals numbered 1975 to 1986, except number 1979, were each inoculated intraperitoneally with 2 cubic centimeters, equal to 2 agar cultures. The guinea pigs comprising this series were small, averaging from 150 to 175 grams in weight. On July 16, two months after the vaccination, the animals which remained alive were all reinoculated in the following manner: A 48-hour agar culture of "Pest Virulent" (from guinea pig number 2174, second transplant) was suspended in 5 cubic centimeters of bouillon and 5 oesen of this suspension rubbed over a small, freshly shaved area on the abdomen and the skin scarified with a knife. Twenty-five controls were inoculated in the same manner and served for this series and for the following one. (Series 37).

Animal No.	Vaccinated intraperitoneally May 16 with—	Reinoculated July 16 with—	Result.	Autopsy and remarks.
1975	Two 48-hour cultures "Pest Avirulent."	5 oesen suspension "Pest Virulent."	Alive and well	
1976	do	do	do	
1977	do	do	do	
1978	do	do	Dead July 21, after 5 days.	Numerous pest bacilli in smears from spleen. Many swollen. Few postmortem bacilli.
1980	do	do	Alive and well	

SERIES 32—Continued.

Animal No.	Vaccinated intraperitoneally May 16 with—	Reinoculated July 16 with—	Result.	Autopsy and remarks.
1981	Two 48-hour cultures "Pest Avirulent."		Not reinoculated; dead May 19, 3 days after vaccination.	No evidence of pest at the post-mortem examination. Peritoneum hyperæmic but no hæmorrhages. In smears from spleen no organisms seen. Cultures from spleen and heart negative. In cultures from the abdominal cavity about 10 colonies developed. Spleen not swollen.
1982	do		Not reinoculated; dead May 17, 23 hours after vaccination.	No evidence of pest. Marked hyperæmia of the abdominal wall. Culture from the heart negative. Culture from the abdominal cavity, fair number of pest bacilli.
1983	do	5 oesen suspension "Pest Virulent."	Alive and well	
1984	do		Not reinoculated; dead May 17, less than 24 hours after vaccination.	No evidence of pest infection. No hæmorrhages in abdomen. Walls slightly œdematous. Culture from the heart negative. From abdominal cavity numerous colonies of pest. 1 cc. of the fluid present in the abdominal cavity was injected into the abdomen of guinea pig No. 1998 which remained alive.
1985	do		Not reinoculated; dead May 22, 6 days after vaccination.	No evidences of pest. Abdominal wall hyperæmic. No hæmorrhage. Spleen not swollen. No fluid in abdomen. In cover slip from the spleen and abdomen no bacilli seen. Culture from abdomen 15 colonies of pest bacilli. In culture from heart, 2 colonies.
1986	do		Not reinoculated; dead May 20, 4 days after vaccination.	Culture from heart negative. Culture from the abdomen, numerous colonies with typical morphology of pest bacillus.

SERIES 32—Continued.

CONTROLS.				
Animal No.	Vaccinated intraperitoneally May 16 with—	Reinoculated July 16 with—	Result.	Autopsy and remarks.
2098		5 oesen suspension "Pest Virulent."	Dead July 21, after 5 days.	Pest. Numerous bacilli in smears from the spleen.
2099		do	Dead July 26, after 10 days.	Do.
2100		do	do	Do.
2101		do	Dead July 23, after 7 days.	Do.
2102		do	Dead July 21, after 5 days.	Do.
2103		do	Dead July 23, after 7 days.	Do.
2104		do	Dead July 19, after $2\frac{1}{3}$ days.	Do.
2105		do	Dead July 26, after 10 days.	Do.
2106		do	Dead July 20, after 4 days.	Do.
2107		do	Dead July 23, after 7 days.	Do.
2108		do	do	Do.
2109		do	do	Do.
2110		do	do	Do.
2111		do	Dead July 20, after 4 days.	Do.
2112		do	Dead July 24, after 8 days.	Do.
2113		do	Dead July 22, after 6 days.	Do.
2114		do	Dead July 23, after 7 days.	Do.
2115		do	do	Do.
2116		do	do	Do.
2117		do	do	Do.
2118		do	do	Do.
2119		do	Dead July 22, after 6 days.	Do.
2120		do	do	Do.
2121		do	Dead July 23, after 7 days.	Do.
2122		do	do	Do.

SERIES 37.—*Inoculations with "Pest Avirulent" in guinea pigs.*

May 20: Ten 24-hour agar cultures of "Pest Avirulent" were suspended in 10 cubic centimeters of normal saline solution (1 cubic centimeter to each culture). Each animal received 1 cubic centimeter intraperitoneally. Some of the guinea pigs of this series did not weigh over 175 grams. One of these apparently succumbed from the effect of the vaccination. On July 16, nearly two months after the vaccination, the animals were all reinoculated with 5 oesen of a suspension of "Pest Virulent" (one 48-hour culture from guinea pig number 2174, second transplant in 5 cubic centimeters of bouillon), rubbed over a freshly shaved area on the abdomen which was scarified with a scalpel. These animals were all inoculated in exactly the same manner and on the same day as the animals comprising Series 32, 33 and 38 (pp. 218 and 219). Twenty-five control animals, numbers 2098 to 2122, were also inoculated, all of which died. The details of the autopsies are given in Series 32 (p. 206).

Animal No.	Immunized May 20 intraperitoneally with—	Reinoculated July 16 with—	Result.	Autopsy and remarks.
2036	One 24-hour culture "Pest Avirulent."		Died June 1, 12 days after vaccination.	No pest.
2037	do	5 oesen suspension "Pest Virulent" over abdomen.	Alive and well	
2038	do	do	Dead July 26, after 10 days.	Typical pest spleen and bubo. Numerous pest bacilli in smears from spleen.
2039	do	do	Alive and well	
2040	do	do	do	
2041	do	do	do	
2042	do	do	do	
2043	do		Small guinea pig, 180 grams, died May 21, less than 24 hours after vaccination.	No post-mortem evidence of pest. No peritonitis or hæmorrhages. Culture from the abdomen developed from 150 to 200 colonies. Evidently the majority of the organisms had perished. Culture from heart sterile.
2044	do	5 oesen suspension "Pest Virulent" over abdomen.	Alive and well	

SERIES 39.—*Inoculations with "Pest Avirulent" in guinea pigs.*

June 10: Fifteen 24-hour agar slant cultures of "Pest Avirulent" were suspended in 15 cubic centimeters of normal saline solution, and each guinea pig was inoculated subcutaneously with 1 cubic centimeter of the suspension. On August 3, nearly two months after the vaccination, the animals were all reinoculated, 5 oesen of a suspension of "Pest Virulent" (one 48-hour culture from guinea pig number 2204, second transplant, in 5 cubic centimeters bouillon) being rubbed over a shaved area on the abdomen which was scarified with a knife. Twenty control guinea pigs were also inoculated at the same time and in the same manner. These animals also served for controls for series number 40 (p. 219).

Animal No.	Inoculated subcutaneously June 10 with—	Reinoculated August 3 with—	Result.	Autopsy and remarks.
2075	One 24-hour agar culture "Pest Avirulent."	5 oesen of suspension of "Pest Virulent" rubbed over abdomen.	Dead Aug. 11, after 8 days	Pest.
2076	do	do	Dead Aug. 12, after 9 days	Do.
2077	do	do	Alive and well	
2078	do	do	do	
2079	do	do	Dead Aug. 10, after 7 days	Typical pest bubo. Innumerable bacilli.
2080	do	do	Alive and well	
2081	do	do	Dead Aug. 8, after 5 days	Innumerable pest bacilli in smears from the spleen.
2082	do	do	Dead Aug. 11, after 8 days	Do.
2083	do	do	Alive and well	
2084	do	do	Dead Aug. 12, after 9 days	Pest.
2085	do	do	Alive and well	
2086	do	do	do	
2087*	do	do	do	
2088	do	do	do	
2089	do	do	Dead Aug. 14, after 11 days	Pest. Typical spleen, containing necrotic foci.

SERIES 39—Continued.

CONTROLS.				
Animal No.	Inoculated subcutaneously June 10 with—	Reinoculated August 3 with—	Result.	Autopsy and remarks.
2249		5 oesen of suspension of "Pest Virulent" rubbed over abdomen.	Dead Aug. 12, after 9 days	Innumerable pest bacilli in smears from spleen.
2250		do	Dead Aug. 11, after 8 days	Do.
2251		do	Dead Aug. 13, after 10 days	Do.
2252		do	Dead Aug. 10, after 7 days	Do.
2253		do	Dead Aug. 14, after 11 days	Do.
2254		do	Dead Aug. 8, after 5 days	Do.
2255		do	Dead Aug. 11, after 8 days	Do.
2256		do	Dead Aug. 8, after 5 days	Do.
2257		do	Dead Aug. 14, after 11 days	Do.
2258		do	Dead Aug. 11, after 8 days	Do.
2259		do	Dead Aug. 7, after 4 days	Do.
2260		do	Dead Aug. 10, after 7 days	Do.
2261		do	Dead Aug. 9, after 6 days	Do.
2262		do	Dead Aug. 13, after 10 days	Do.
2263		do	do	Do.
2264		do	Dead Aug. 11, after 8 days	Do.
2265		do	do	Do.
2266		do	Dead Aug. 10, after 7 days	Do.
2267		do	Dead Aug. 9, after 6 days	Do.
2268		do	Dead Aug. 13, after 10 days	Do.

*Animal was pregnant at the time of vaccination and gave birth to two healthy young 7 days after its reinoculation.

SERIES 41.—*Inoculations with "Pest Avirulent" in guinea pigs.*

June 30: Twenty-one 24-hour agar slant cultures of "Pest Avirulent" were suspended in 21 cubic centimeters of saline solution and each guinea pig was inoculated subcutaneously with 1 cubic centimeter of this suspension. On August 28, two months after the vaccination, the animals were all reinoculated with 5 oesen of a suspension of "Pest Virulent" (two 48-hour cultures from guinea pig number 2315, second generation, in 10 cubic centimeters bouillon) being rubbed over a freshly shaved and scarified area on the abdomen. Twenty-five control animals were also inoculated at the same time and in the same manner.

Animal No.	Vaccinated June 30 subcutaneously with—	Reinoculated August 28 with—	Result.	Autopsy and remarks.
2146	One 24-hour culture "Pest Avirulent."	5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Alive and well	
2147	do	do	Dead Sept. 4, after 7 days	Pest.
2148	do	do	Dead Sept. 6, after 9 days	Moderate-sized bubo and abscesses in the spleen.
2149	do	do	Alive and well	
2150	do	do	do	

SERIES 41—Continued.

Animal No.	Vaccinated June 30 subcutaneously with—	Reinoculated August 28 with—	Result.	Autopsy and remarks.
2151	One 24-hour culture "Pest Avirulent."	5 oesen suspension "Pest Virulent" rubbed over abdomen.	Alive and well	
2152	do	do	do	
2153	do	do	do	
2154	do	do	do	
2155	do	do	Dead Sept. 7, after 10 days	Large bubo. Miliary abscesses in spleen.
2156	do	do	Alive and well	
2157	do	do	do	
2158	do	do	do	
2159	do	do	do	
2160	do	do	Dead Sept. 12, after 15 days	Pest bubo. Very few bacilli in the pus from the bubo.
2161	do	do	Alive and well	
2162	do	do	do	
2263	do	do	Dead Sept. 7, after 10 days	Bubo. Spleen apparently normal. No bacilli in smears.
2164	do	do	Alive and well	
2165	do	do	Dead Sept. 7, after 10 days	Large bubo. Abscess in the spleen.
2166	do	do	Alive and well	
CONTROLS.				
2362		5 oesen suspension "Pest Virulent rubbed over abdomen.	Dead Sept. 4, after 7 days	Numerous pest bacilli in smears from spleen.
2363		do	do	Do.
2364		do	do	Do.
2365		do	do	Do.
2366		do	Dead Sept. 2, after 5 days	Do.
2367		do	Dead Sept. 4, after 7 days	Do.
2368		do	Dead Sept. 2, after 5 days	Do.
2369		do	do	Do.
2370		do	do	Do.
2371		do	Dead Sept. 4, after 7 days	Do.
2372		do	Dead Sept. 2, after 5 days	Do.
2373		do	Dead Sept. 12, after 15 days	Do.
2374		do	Dead Sept. 2, after 5 days	Do.
2375		do	Dead Sept. 8, after 11 days	Do.
2376		do	Dead Sept. 4, after 7 days	Do.
2377		do	Dead Sept. 6, after 9 days	Do.
2378		do	Dead Sept. 2, after 5 days	Do.
2379		do	Dead Sept. 4, after 7 days	Do.
2380		do	Dead Sept. 6, after 9 days	Do.
2381		do	Dead Sept. 2, after 5 days	Do.
2382		do	Dead Sept. 4, after 7 days	Do.
2383		do	do	Do.
2384		do	do	Do.
2385		do	Dead Sept. 6, after 9 days	Do.
2386		do	Dead Sept. 8, after 11 days	Do.

SERIES 46.—*Inoculations with "Pest Avirulent" in guinea pigs.*

October 26: Fifteen 48-hour agar slant cultures of the strain "Pest Avirulent" were suspended in 15 cubic centimeters of saline solution (1 cubic centimeter to each culture). Each guinea pig of the series was inoculated subcutaneously with 1 cubic centimeter of this suspension. On November 26, one month after the vaccination, the immunity of the animals was tested in the following manner:

Five oesen of a suspension of "Pest Virulent" (two 48-hour cultures from guinea pig number 2858, second transplant in 10 cubic centimeters of bouillon) being rubbed over a freshly shaved but not scarified area of the abdomen. Twenty-five control animals were also inoculated at the same time and in the same manner. The immunity of the animals of this series was tested on the same date and with exactly the same suspension as was that of the animals comprising Series 47, 50, and 54. (See pp. 220, 197, and 232.)

Animal No.	Vaccinated October 26 subcutaneously with—	Reinoculated November 26 with—	Result.	Autopsy and remarks.
2633	One 24-hour culture "Pest Avirulent."	5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Dead Dec. 4, after 8 days	Typical pest buboes and spleen.
2634	do	do	Alive and well	
2635	do	do	Dead Dec. 8, after 12 days	Old bubo and few abscesses in spleen.
2636	do	do	Alive and well	
2637	do	do	do	
2638	do	do	do	
2639	do	do	do	
2640	do	do	do	
2641	do	do	Dead Dec. 3, after 7 days	Large bubo. Spleen contains few abscesses.
2642	do	do	Alive and well	
2643	do	do	do	
2644	do	do	do	
2645	do	do	do	
2646	do	do	do	
2647	do	do	do	
		CONTROLS.		
2870		5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Dead Dec. 3, after 7 days	Typical bubo. Numerous pest bacilli in smears from spleen.
2871		do	Dead Dec. 1, after 5 days	Do.
2872		do	Dead Dec. 3, after 7 days	Do.
2873		do	Dead Dec. 2, after 6 days	Do.
2874		do	Dead Dec. 4, after 8 days	Do.
2875		do	Dead Dec. 1, after 5 days	Do.
2876		do	Dead Nov. 30, after 4 days	Do.
2877		do	Dead Dec. 2, after 6 days	Do.
2878		do	Dead Nov. 30, after 4 days	Do.
2879		do	Dead Dec. 5, after 9 days	Do.

SERIES 46.—Continued.

CONTROLS—Continued.

Animal No.	Vaccinated October 26 subcutaneonsly with—	Reinoculated November 26 with—	Result.	Autopsy and remarks.
2880		5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Dead Dec. 4, after 8 days	Typical bubo. Numerous pest bacilli in smears from spleen.
2881		do	Dead Dec. 7, after 11 days	Do.
2882		do	Dead Nov. 30, after 4 days	Do.
2883		do	do	Do.
2884		do	Dead Dec. 2, after 6 days	Do.
2885		do	Dead Dec. 1, after 5 days	Do.
2886		do	Dead Nov. 30, after 4 days	Do.
2887		do	Dead Dec. 1, after 5 days	Do.
2888		do	do	Do.
2889		do	Dead Nov. 30, after 4 days	Do.
2890		do	Dead Dec. 1, after 5 days	Do.
2891		do	Dead Nov. 30, after 4 days	Do.
2892		do	Dead Dec. 3, after 7 days	Do.
2893		do	Dead Dec. 1, after 5 days	
2894		do	do	

SERIES 17.—*Inoculations with the strain "Maassen Alt" in monkeys.*

November 3: Six 48-hour agar slant cultures of "Maassen Alt" were suspended in 7 cubic centimeters of bouillon. Animals numbered 1369 to 1371 were each inoculated subcutaneously with 2 cubic centimeters of this suspension which equals nearly 2 agar slants, and animal number 1372 with 1 cubic centimeter, or nearly 1 agar slant of "Maassen Alt." On November 23, twenty days after the vaccination, 2 agar slant cultures of "Pest Virulent" (from guinea pig number 1452, second transplant) were suspended in 10 cubic centimeters of bouillon and each animal was reinoculated subcutaneously, on the opposite side of the body to that upon which previous inoculation had been performed, with 0.25 cubic centimeter of this suspension, which is equal to ½ oese "Pest Virulent." Eleven control animals were at the same time also inoculated subcutaneously with the same dose.

Animal No.	Vaccinated November 3 subcutaneously with—	Infected November 23 with—	Result.	Autopsy and remarks.
1369	Nearly 2 agar slants "Maassen Alt."	½ oese "Pest Virulent."	Alive and well	
1370	do	do	do	
1371	do	do	do	
1372	Nearly 1 agar slant "Maassen Alt."	do	Dead Nov. 29, after 6 days.	Innumerable pest bacilli in smears from the spleen.

SERIES 17—Continued.

CONTROLS.

Animal No.	Vaccinated November 3 subcutaneously with—	Infected November 23 with—	Result.	Autopsy and remarks.
1484		⅛ oese "Pest Virulent."	Dead Nov. 28, after 5 days.	Innumerable pest bacilli in smears from the spleen.
1485		do	do	Do.
1486		do	do	Do.
1487		do	Dead Nov. 27, after 4 days.	Do.
1488		do	do	Do.
1489		do	do	Do.
1490		do	Dead Nov. 28, after 5 days.	Do.
1491		do	Dead Nov. 27, after 4 days.	Do.
1492		do	do	Do.
1493		do	do	Do.
1494		do	do	Do.

SERIES 21.—*Inoculations with the strain "Maassen Alt" in monkeys.*

November 14: Twenty-one 48-hour agar slant cultures of "Maassen Alt" were suspended in 21 cubic centimeters of bouillon. Monkeys numbered 1438 to 1445 inclusive each received 2 cubic centimeters subcutaneously, which is equal to 2 agar slant cultures, and monkeys numbered 1446 to 1449 inclusive, 1 cubic centimeter subcutaneously, equal to 1 agar slant culture. On November 28, two weeks after the vaccination, the animals were all reinoculated in the following manner. Two agar slant cultures of "Pest Virulent" (from guinea pig number 1479, second transplant) were suspended in 10 cubic centimeters of bouillon and each animal was inoculated subcutaneously on the opposite side of the body to which the vaccination had been made with 0.25 cubic centimeter of this suspension, equal to ½ oese "Pest Virulent." Twelve control animals were all inoculated at the same time with the same dose of this suspension, except animals numbered 1503 and 1504, which were very large monkeys and which received 0.33 cubic centimeter.

Animal No.	Vaccinated November 14 subcutaneously with—	Infected November 28 with—	Result.	Autopsy and remarks.
1438	2 agar slant cultures.	½ oese "Pest Virulent."	Alive and well	
1439	do	do	Dead Dec. 5, after 7 days.	Very few pest bacilli in smears from the spleen and near point of inoculation. Tissues near point of inoculation reddened and œdematous.
1440	do	do	Alive and well	
1441	do	do	do	
1442	do	do	Dead Dec. 6, after 8 days.	A few bacilli in smears from the spleen. Innumerable in ulcerating bubo which also contains numerous cocci.

SERIES 21—Continued.

Animal No.	Vaccinated November 14 subcutaneously with—	Infected November 28 with—	Result.	Autopsy and remarks.
1443	2 agar slant cultures.	½ oese "Pest Virulent."	Alive and well	
1444	do	do	do	
1445	do	do	do	
1446	1 agar slant culture.	do	do	
1447	do	do	Dead Dec. 4, after 6 days.	Pest bacilli fairly numerous in smears from the spleen. No appearance of bubo about point of inoculation although pest bacilli are numerous in the tissues in this region.
1448	do	do	Dead Dec. 18, after 10 days.	A large sloughing abscess at the point of inoculation in which a fair number of pest bacilli and streptococci are present. Smears from spleen show a few bacilli.
1449	do	do	Alive and well	
CONTROLS.				
1499		½ oese "Pest Virulent."	Dead Dec. 2, after 4 days.	Innumerable pest bacilli in smears from the spleen.
1500		do	Dead Dec. 3, after 5 days.	Do.
1501		do	Dead Dec. 1, after 3 days.	No bacilli found in one smear from spleen. At point of inoculation a hæmorrhagic bubo is present in which numerous pest bacilli are present.
1502		do	do	Very numerous pest bacilli in smears from spleen.
1503		⅔ oese "Pest Virulent."	Dead Dec. 2, after 4 days.	Innumerable pest bacilli in smears from spleen.
1504		do	Dead Dec. 4, after 6 days.	Do.
1505		½ oese "Pest Virulent."	Dead Dec. 3, after 5 days.	Fairly numerous pest bacilli in smears from spleen.
1506		do	Dead Dec. 1, after 3 days.	Numerous pest bacilli in smears from spleen.
1507		do	Dead Dec. 3, after 5 days.	Fairly numerous pest bacilli in smears from spleen.
1508		do	Dead Dec. 2, after 4 days.	Innumerable pest bacilli in smears from spleen.
1509		do	do	Do.
1510		do	Dead Dec. 6 after 8 days.	Very numerous pest bacilli in smears from spleen.

SERIES 24.—*Inoculations with the strain "Maassen Alt" in monkeys.*

On December 4, thirty-two, 48-hour agar slant cultures of "Maassen Alt" were suspended in 32 cubic centimeters of bouillon (1 cubic centimeter to each tube). Monkeys numbered 1536 to 1547 inclusive were inoculated subcutaneously each with 2 cubic centimeters of this suspension, equal to 2 agar cultures; monkeys numbered 1548 to 1552 inclusive with 1 cubic centimeter each and monkey number 1553 with a little less than ½ cubic centimeter, equal to about ½ culture. Four of the animals succumbed, apparently from the result of the vaccination. On December 18 the surviving ones were all reinoculated in the following manner: A 48-hour agar slant culture of "Pest Virulent" (from guinea pig number 1516, second transplant) was suspended in 5 cubic centimeters of bouillon, and each animal inoculated subcutaneously on the opposite side of the body to that upon which the first inoculation was given with 0.2 cubic centimeter, which equals a little less than ½ oese of "Pest Virulent." Attention must be called to the fact that this series was inoculated exactly the same as Series 23. Among the animals comprising the present Series 24 there were also a number of large monkeys. For controls, the same animals given as controls in Series 23 (see p. 191), numbered 1594 to 1605, were employed, all being inoculated in the same manner and on the same day. It will be seen from the table that a number of the controls did not die and hence conclusions regarding the exact value of the immunization in this series can not be drawn.

Animal No.	Vaccinated December 4 subcutaneously with—	Infected December 18 with—	Result.	Autopsy and remarks.
1536	2 agar slant cultures, "Maassen Alt."	----------------	Dead Dec. 7, 3 days after first inoculation.*	Skin burned and incised at point of inoculation revealing an abscess containing pus in which numerous organisms are present. Smears from spleen are negative. Cultures from the spleen developed one colony of *Staphylococcus aureus*. Culture from abscess shows very numerous colonies of *Staphylococcus aureus* and also of "Maassen Alt."
*1537	-----do--------	----------------	Dead Dec. 5, one day after first inoculation.*	Cultures from the point of inoculation show few colonies of "Maassen Alt" and of *Staphylococcus* aureus. Culture from the spleen negative.
1538	-----do--------	Nearly ½ oese "Pest Virulent."	Alive and well.	

*It is not entirely clear that these animals succumbed to a pest infection alone. They were evidently in poor health and in three the *Staphylococcus aureus* was also isolated. In the case of monkey number 1552 it seems clear, however, that the animal really succumbed to pest infection. It must be noted that animals numbered 1536, 1537 and 1547 died in a much shorter time than one would expect from a pest infection with an avirulent organism.

SERIES 24—Continued.

Animal No.	Vaccinated December 4 subcutaneously with—	Infected December 18 with—	Result.	Autopsy and remarks.
1539	2 agar slant cultures, "Maassen Alt."	Nearly ½ oese "Pest Virulent."	Alive and well.	
1540	do	do	do	
1541	do	do	do	
1542	do	do	do	
1543	do	do	do	
1544	do	do	do	
1545	do	do	Dead Dec. 21, after 3 days.	Animal considerably emaciated. No organisms found in spleen. Cultures and smears from the tissues near the point of inoculation show numerous pest bacilli.
1546	do	do	Alive and well.	
*1547	do		Died from first inoculation Dec. 7, after 3 days.*	Skin burned and incised, revealing a small abscess in which numerous pest bacilli ("Maassen Alt") are present. Cultures developed numerous colonies of this organism and of *Staphylococcus aureus*.
1548	1 agar slant culture, "Maassen Alt."	Nearly ½ oese "Pest Virulent."	Alive	
1549	do	do	do	
1550	do	do	do	
1551	do	do	do	
*1552	do		Died from first inoculation Dec. 10, after 6 days.*	Nothing apparently abnormal about the point of inoculation. No abscess or pest bacilli seen in smears. In 2 cultures from the spleen, one developed 12 colonies and the other 14 colonies of "Maassen Alt."
1553	About ½ agar slant culture "Maassen Alt."	Nearly ½ oese "Pest Virulent."	Alive	

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* It is not entirely clear that these animals succumbed to a pest infection alone. They were evidently in poor health and in three the *Staphylococcus aureus* was also isolated. In the case of monkey number 1552 it seems clear, however, that the animal really succumbed to pest infection. It must be noted that animals numbered 1536, 1537 and 1547 died in a much shorter time than one would expect from a pest infection with an avirulent organism.

Series 52.—*Inoculations with the strain "Maassen Alt" in monkeys.*

Twenty-two 48-hour agar slant cultures of the strain "Maassen Alt" were suspended in 22 cubic centimeters saline solution (1 cubic centimeter to each culture). Monkeys numbered 2721 to 2728 received subcutaneously each 2 cubic centimeters, equal to 2 agar slant cultures. Monkeys numbered 2729 to 2735 each received 1 cubic centimeter, equal to 1 agar slant culture. On November 28, one month after the vaccination, the immunity of the animals was tested by the subcutaneous injection of the strain "Pest Virulent" in exactly the same manner as was the immunity of the animals in Series 48 and 51. (See pp. 195 and 203.) Twenty-five control animals, numbered 2896 to 2920, were also inoculated in the same manner with the same suspension and succumbed to acute infection. For the details of the protocols see Series 48.

Animal No.	Vaccinated October 26 subcutaneously with—	Infected November 28 with—	Result.	Autopsy and remarks.
2721	2 agar cultures strain "Maassen Alt."	⅔ oese strain "Pest Virulent."	Alive and well	
2722	do		Dead Nov. 8, after vaccination.	No evidence of bubo or inflammation at point of inoculation. Cover slips no excess of leucocytes, no bacteria. Cultures from spleen and point of inoculation negative. Three cultures. Large amounts material inoculated.
2723	do	⅔ oese strain "Pest Virulent."	Alive and well	
2724	do	do	Dead Dec. 4, after 6 days.	Numerous pest bacilli in smears from spleen.
2725	do	do	do	Bubo with numerous pest bacilli.
2726	do	do	Alive and well	
2727	do	do	do	
2728	do	do	Dead Dec. 1, after 3 days.	Numerous pest bacilli in smears from spleen.
2729	1 agar culture strain "Maassen Alt."	do	Alive and well	
2730	do	do	Dead Dec. 3, after 5 days.	Bubo. Numerous pest bacilli in smears from spleen.
2731	do	do	Alive and well	
2732	do	do	do	
2733	do	do	Dead Dec. 3, after 5 days.	Bubo. Few pest bacilli.
2734	do	do	Dead Dec. 1, after 3 days.	Innumerable pest bacilli in smears from spleen.
2735	do	do	Dead Dec. 2, after 2 days.	Bubo. Fair numbers of pest bacilli in smears from spleen.

Series 33.—*Inoculations with the strain "Maassen Alt" in guinea pigs.*

May 16: Twenty-two 48-hour agar slant cultures of the strain "Maassen Alt" were suspended in 22 cubic centimeters of saline solution (1 cubic centimeter to each culture). Guinea pigs numbered 1987 to 1996 were inoculated intraperitoneally each with 1 cubic centimeter. On July 16, two months after the vaccination, the animals were all reinoculated with the strain "Pest Virulent" in exactly the same manner as the animals in Series 32, 37 and 38 (see pp. 204, 207 and 219), the same 25 control animals, numbered 2098 to 2122, all of which died of pest, serving for all three of these series.

Animal No.	Vaccinated intraperitoneally May 16 with—	Infected July 16 with—	Result.	Autopsy and remarks.
1987	1 cc. "Maassen Alt."	5 oesen "Pest Virulent rubbed over freshly shaved area on abdomen and skin lightly scarified with a knife.	Alive and well.	
1988	do	do	do	
1989	do	do	do	
1990	do	do	do	
1991	do	do	do	
1992	do		Dead May 17, about 21 hours after vaccination.	A few hæmorrhages in the abdominal walls. No fluid. Culture from the abdominal cavity shows a fair number of pest bacilli.
1993	do	5 oesen "Pest Virulent" rubbed over freshly shaved area on abdomen and skin lightly scarified with a knife.	Alive and well.	
1994	do		Dead May 17, 23 hours after vaccination.	No evidences of pest infection. Little fluid in the abdominal cavity, culture from heart negative. In culture from abdominal cavity a fair number of pest bacilli developed.
1995	do		Dead May 18, 2 days after vaccination.	Cultures from heart sterile. Culture from abdomen, a rich growth of pest.
1996	do	5 oesen "Pest Virulent" rubbed over freshly shaved area on abdomen and skin lightly scarified with a knife.	Alive and well.	

SERIES 38.—*Inoculation with the strain "Maassen Alt" in guinea pigs.*

May 20: Eight 24-agar slant cultures of "Maassen Alt" were suspended in 8 cubic centimeters of normal saline solution (1 cubic centimeter to each culture) and each animal was inoculated intraperitoneally with 1 cubic centimeter of the suspension. One animal succumbed from the effect of the vaccination. On July 16, about two months after the vaccination, the animals were all reinoculated, 5 oesen of the same suspension of "Pest Virulent" employed in reinoculating Series 32, 33 and 37 being rubbed over a shaved and scarified area on the abdomen. The same twenty-five control animals, numbered 2098 to 2122, employed in Series 32 and 33 (see pp. 204 and 218), which all died, answered for controls for the present series.

Animal No.	Vaccinated May 20, intraperitoneally with—	Infected July 16 with—	Result.	Autopsy and remarks.
2045	One 24-hour culture "Maassen Alt."	5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Alive and well	
2046	do	do	Dead July 31, after 15 days.	Very few pest bacilli in smears from the spleen. Small bubo.
2047	do	do	Alive and well	
2048	do	do	do	
2049	do	do	do	
2050	do		Dead May 22, 2 days after vaccination.	Culture from heart and spleen negative. From the abdomen about 100 colonies of "Maassen Alt."
2051	do	5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Dead July 21, after 5 days.	Fair number of pest bacilli and numerous post-mortem invading bacilli in smears from spleen.

SERIES 40.—*Inoculations with the strain "Maassen Alt" in guinea pigs.*

June 15: Fifteen 24-agar slant cultures of "Maassen Alt" were suspended in 15 cubic centimeters saline solution, and each guinea pig inoculated subcutaneously with 1 cubic centimeter of this suspension. On August 3, nearly one and one-half months after the vaccination, the animals were all but one reinoculated, 5 oesen of a suspension of "Pest Virulent" (one 48-hour culture from guinea pig number 2204, second transplant, in 5 cubic centimeters bouillon) being rubbed over a shaved area on the abdomen which was lightly scarified with a knife. It is to be noticed that these animals were all inoculated in exactly the same manner as those described in Series 39. The same twenty control animals, numbered 2249 to 2268 (see p. 209), which all died, also served for the present series.

SERIES 40—Continued.

Animal No.	Vaccinated subcutaneously June 15 with—	Infected August 3 with—	Result.	Autopsy and remarks.
2125	One 24-hour culture "Maassen Alt."	5 oesen of suspension of "Pest Virulent" rubbed over abdomen.	Alive and well	
2126	do	do	do	
2127	do	do	do	
2128	do		Dead June 16, less than 24 hours after vaccination.	Culture from heart and spleen negative. Cover slip from point of inoculation shows a few degenerating bacilli. Animal evidently died of plague toxæmia.
2129	do	5 oesen of suspension of "Pest Virulent" rubbed over abdomen.	Alive and well	
2130	do	do	do	
2131	do	do	Dead Aug. 14, after 11 days.	Few pest bacilli in smears from spleen.
2132	do	do	Alive and well	
2133	do	do	Dead Aug. 12, after 9 days.	Pest.
2134	do	do	Alive and well	
2135	do	do	do	
2136	do	do	do	
2137	do	do	do	
2138	do	do	Dead Aug. 13, after 10 days.	Pest.
2139	do	do	Alive and well	

SERIES 47.—*Inoculations with the strain "Maassen Alt" in guinea pigs.*

October 26: Fifteen 48-hour agar slant cultures of the strain "Maassen Alt" were suspended in 15 cubic centimeters of saline solution (1 cubic centimeter to each culture). Each guinea pig of the series was inoculated subcutaneously with 1 cubic centimeter of the suspension equal to 1 agar slant culture. On November 26, about one month after the vaccination, the immunity of the animals was tested in the following manner:

Five oesen of a suspension of "Pest Virulent" (two 48-hour cultures from guinea pig number 2858, second transplant, in 10 cubic centimeters of bouillon) being rubbed over the freshly shaved but not scarified area of the abdomen. Twenty-five control animals, numbered 2870 to 2894, were also inoculated with the virulent organism at the same time and in the same manner. All of these succumbed to acute plague infection. For the details of the protocols see Series 46 (p. 211).

SERIES 17—Continued.

The immunity of the animals in this series was tested in the same manner and with exactly the same suspension as was used for the animals comprising Series 46, 50, and 54. (See pp. 211, 197, and 232.)

Animal No.	Vaccinated October 26 subcutaneously with—	Infected November 26 with—	Result.	Autopsy and remarks.
2648	One 24-hour agar culture "Maassen Alt."	5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Alive and well.	
2649	do	do	do	
2650	do	do	do	
2651	do	do	do	
2652	do	do	do	
2653	do		Died Oct. 27, 1 day after vaccination.	No plague bacilli found in cover slips from abdominal cavity or from point of inoculation. Some fluid had entered abdominal cavity. Animal evidently died of toxæmia.
2654	do	5 oesen suspension of "Pest Virulent" rubbed over abdomen.	Alive and well.	
2655	do	do	do	
2656	do	do	do	
2657	do	do	do	
2658	do	do	do	
2659	do	do	do	
2660	do	do	do	
2661	do	do	do	
2662	do	do	do	

EXPERIMENTS IN THE INOCULATION OF ANIMALS WITH EXTRACTS OF THE STRAIN "PEST VIRULENT."

SERIES 7.—*Inoculations in monkeys with extracts of the strain "Pest Virulent" (free receptors).*

Twenty-one, 48-hour slant cultures of the strain "Pest Virulent" were suspended in 21 cubic centimeters of saline solution. The organisms were killed by heating for one hour at 60° C. and the suspension placed in the incubator at 37° C. for three days. At the end of this time it was filtered through a Berkefeld candle. The animals were inoculated subcutaneously with the filtrate as shown below. Ten days later their immunity was tested by sticking them with a 10 cubic centimeter syringe needle which had been dipped in a suspension of "Pest Virulent" (one 48-hour agar slant "Pest Virulent," guinea pig number 1243, second transplant) suspended in 5 cubic centimeters of bouillon.

SERIES 7—Continued.

Animal No.	How immunized.	How infected.	Result.	Autopsy and remarks.
1242	5 cc. autolytic product = 50 mg. of culture.	Stuck with infected 10 cc. syringe needle.	Dead after 7 days.	Innumerable pest bacilli in spleen.
1245	do	do	Dead after 6 days.	Pest septicæmia. Numerous organisms in the heart's blood.
1246	do	do	Dead after 11 days.	Numerous organisms in the spleen.
1247	4.8 cc. autolytic product = 48 mg. of culture.	do	Alive	
CONTROLS.				
1293		Stuck, with infected 10 cc. syringe needle.	Dead after 4 days.	Smears from spleen and heart negative for organisms. Culture from heart, *Staphylococcus aureus*. Pest organism probably still localized near point of inoculation.
1294		do	Dead after 7 days.	No pest bacilli found in the smears from the spleen. Very numerous in smears from the tissues near the point of inoculation.
1295		do	Dead after 4 days.	Innumerable pest bacilli in smears from spleen. Cultures from heart show *B. pestis* (pure).

SERIES 26.—*Inoculations in monkeys with extracts of the strain "Pest Virulent" (free receptors).*

On December 8 sixteen 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1514, second generation) were suspended in 16 cubic centimeters of distilled water, the organisms killed by heating at 60° C. for one hour and the suspension then placed in the incubator at 37° C. for four days. At the end of this time it was filtered through a Berkefeld candle. The suspension both before and after filtration was sterile. On December 12 the animals of this series were inoculated subcutaneously with the filtrate as below. On December 26, fourteen days after the first inoculation, the animals were infected in the following manner: Six 48-hour agar slant cultures of "Pest Virulent" (from guinea pig number 1592, second transplant) were suspended in 30 cubic centimeters of bouillon (5 cubic centimeters to each culture). Each animal was inoculated subcutaneously on the opposite side of the body with 0.33 cubic centimeters, equal to ⅔ oese, of "Pest Virulent." Twelve controls, numbered 1613 to 1624, were all inoculated at the same time with the same dose. The details of the results with the controls may be seen in Table No. 25. (See p. 194.) All of them succumbed to acute pest infection.

SERIES 26—Continued.

Animal No.	Inoculated December 12 subcutaneously with—	Reinoculation December 26 with—	Result.	Autopsy and remarks.
1583	Free receptors extracted from 5 agar slant cultures "Pest Virulent."	⅔ oese "Pest Virulent.	Dead Jan. 13, after 17 days.	Few pest bacilli in smears from spleen. Innumerable organisms in a large bubo over which the skin is not yet broken.
1584	do	do	Alive and well.	
1585	Free receptors extracted from 2 agar slant culture "Pest Virulent."	do	Dead Jan. 2, after 7 days.	Numerous pest bacilli in smears from spleen.
1586	Free receptors extracted from 1 agar slant culture "Pest Virulent."	do	Dead Dec. 31, after 5 days.	Very numerous pest bacilli in smears from the spleen.
1587	do	do	Dead Jan. 1, after 6 days.	Very few pest bacilli in smears from the spleen. Organisms very numerous in smears from bubo.

SERIES 29.—*Inoculations with artificial pest aggressin in monkeys and guinea pigs.*

On January 27, large test tubes of agar were inoculated with first transplant cultures of "Pest Virulent" from guinea pigs numbered 1700, 1698, 1697 and 1676, all of which animals died between the 23d to the 25th of January. The cultures were placed at 30° C. for two days. The growth was then suspended in distilled water, 1 cubic centimeter being used for each culture containing about 30 milligrams of the growth. The whole was mixed and placed on an electrical shaking machine for five days. The suspension was then heated for one and one-half hours at a temperature of 44° to 45° C. and 5 per cent carbolic acid added in sufficient quantity to form a 0.5 per cent solution. After one and one-half days the suspension was thoroughly centrifuged for four hours at a velocity of 4,000 revolutions per minute and the clear fluid above pipetted and used for the inoculations on February 7 as given below. Cultures showed this fluid to be sterile. On February 24 and 25, nearly three weeks after the first inoculation, the animals were reinoculated in the following manner: In the case of the guinea pigs, the abdomen of each was freshly shaved, the skin scarified with a knife, and a portion of the spleen of guinea pig number 1766, which had succumbed this date to acute pest infection, was rubbed well over the scarified area. Three control guinea pigs were inoculated in the same manner. The monkeys were inoculated subcutaneously with 0.33 cubic centimeter of a suspension of "Pest Virulent" (5 cubic centimeters of bouillon to one 48-hour agar slant culture from guinea pig number 1764, second generation, equal to about ⅔ oese.

Inoculations in guinea pigs.

Animal No.	Inoculated February 7 with—	Infected February 24 with—	Result.	Autopsy and remarks.
1731	5 cc. artificial aggressin intraperitoneally.	Abdomen massaged with portion of pest spleen.	Dead Mar. 1, after 5 days.	Typical buboes containing numerous pest bacilli.
1732	do	do	do	Do.

SERIES 29—Continued.

CONTROLS.

Animal No.	Inoculated February 7 with—	Infected February 24 with—	Result.	Autopsy and remarks.
1770		Abdomen massaged with portion of pest spleen.	Dead Feb. 27, after 3 days.	Innumerable pest bacilli, buboes, etc.
1771		do	Dead Mar. 1, after 5 days.	Do.
1772		do	Dead Mar. 5, after 9 days.	Do.

Inoculations in monkeys.

Animal No.	Inoculated February 7 with—	Infected February 25 with—	Result.	Autopsy and remarks.
1733	5 cc. artificial aggressin subcutaneously.	About ⅔ oese "Pest Virulent" subcutaneously.	Dead Mar. 2, after 5 days.	Bubo containing pus and typical pest bacilli.
1734	do	do	Alive and well.	
1735	do	do	Dead Mar. 1, after 4 days.	Very numerous pest bacilli in smears from spleen.
1736	do	do	Dead Feb. 28, after 3 days.	Innumerable pest bacilli in smears from spleen.
1737	do	do	Dead Mar. 3, after 6 days.	Numerous pest bacilli in smears from spleen.
1738	do	do	Dead Mar. 1, after 4 days.	Fair numbers of pest bacilli in smears from spleen.
1739	2.5 cc. artificial aggressin subcutaneously.	do	Dead Mar. 3, after 6 days.	Numerous pest bacilli in smears from spleen.
1740	do	do	Dead Feb. 28, after 3 days.	Smears from spleen negative for pest bacilli. Smears from point of inoculation show innumerable pest bacilli.
1741	do	do	Dead Mar. 1, after 4 days.	Innumerable pest bacilli in smears from spleen.
1742	do	do	Dead Mar. 2, after 5 days.	Œdema and hæmorrhage about point of inoculation. Numerous pest bacilli.
1743	1 cc. artificial aggressin subcutaneously.	do	Dead Mar. 3, after 6 days.	Numerous pest bacilli in smears from spleen.
1744	do	do	Dead Feb. 28, after 3 days.	Innumerable pest bacilli in smears from the spleen.

SERIES 29—Continued.

Animal No.	Inoculated February 7 with—	Infected February 25 with—	Result.	Autopsy and remarks.
1773	5 cc. artificial aggressin subcutaneously.	About ⅔ oese "Pest Virulent" subcutaneously.	Dead Feb. 28, after 3 days.	Innumerable pest bacilli in smears from the spleen.
1774	do	do	do	Do.
1775	do	do	Dead Feb. 27, after 2 days.	Do.
1776	do	do	Dead Feb. 28, after 3 days.	Do.
1777	do	do	do	Do.
1778	do	do	do	Do.
1779	do	do	Alive and well.	
1780	do	do	Dead Feb. 28, after 3 days.	Do.
1781	do	do	do	Do.
1782	do	do	do	Do.
1783	do	do	Alive and well.	
1784	do	do	do	
1785	do	do	Dead Feb. 28, after 3 days.	Do.
1786	do	do	do	Do.
1787	do	do	Dead Mar. 1, after 4 days.	Do.
1788	do	do	Dead Feb. 28, after 3 days.	Do.
1789	do	do	do	Do.
1790	do	do	Dead Mar. 1, after 4 days.	Do.
1791	do	do	Dead Mar. 3, after 6 days.	Do.
1792	do	do	Dead Mar. 1, after 4 days.	Do.
CONTROLS.				
1793		About two-third oese "Pest Virulent" subcutaneously.	Dead Feb. 27, after 2 days.	Do.
1794		do	do	Do.
1795		do	Dead Feb. 28, after 3 days.	Do.
1796		do	do	Do.
1797		do	do	Do.

SERIES 30.—*Inoculations with artificial pest aggressin in guinea pigs.*

February 11: The animals of this series were inoculated with Artificial Aggressin I, details of the preparation of which is given in Series 29. The aggressin tested just before inoculation proved to be sterile. The animals were reinoculated on February 26 by the method described for the guinea pigs in Series 29. (See p. 223.)

Animal No.	How inoculated.	Reinoculated February 26.	Result.	Autopsy and remarks.
1753	4.5 cc. Artificial Aggressin I intraperitoneally Feb. 11.	Abdomen shaved and scarified and massaged with portion of spleen of guinea pig No. 1768 which died this day of plague.	Dead Mar. 1, after 13 days.	Typical pest buboes.
1754	do	do	do	Hæmorrhagic buboes. Numerous pest bacilli in smears.
CONTROLS.				
1798		Abdomen shaved and scarified and massaged with portion of spleen of guinea pig No. 1768 which died this day of plague.	Dead Mar. 1, after 3 days.	Pest. Typical buboes.
1799		do	do	Do.
1800		do	Dead Mar. 2, after 4 days.	Pest bacilli in smears from spleen.

SERIES 31.—*Inoculations with artificial pest aggressin in guinea pigs.*

May 11: Thirty-six 48-hour, large, agar slant cultures * (from second generation, guinea pig number 1956) were suspended in 50 cubic centimeters of distilled water. The suspension was then placed on an electrical shaking machine and was thoroughly shaken for three days. On May 14 it was removed and cultures taken which later developed a pure growth of pest bacilli. Five per cent carbolic acid was then added in sufficient quantity to form a 0.5 per cent solution. The suspension was then centrifuged for five hours at a velocity of 4,000 revolutions per minute and the clear fluid above, pipetted from the bacterial sediment. Cultures from this fluid remained sterile. The animals were inoculated as below. On June 11, one month after the first injection, the immunity of the animals was tested in the following manner: One 48-hour agar slant culture of "Pest Virulent" (from guinea pig number 2067, second transplant) was suspended in 5 cubic centimeters of bouillon, and 5 oesen of this suspension then rubbed over

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* The culture tubes designated in these experiments as "large" contain a surface of agar of more than twice the area of that in the tubes ordinarily employed in bacteriological work.

SERIES 31—Continued.

a shaved area on the abdomen and the skin lightly scarified with a scalpel. Five control animals were all inoculated in the same manner.

Animal No.	Inoculated May 14 with—	Reinoculated June 11 with—	Result.	Autopsy and remarks
1969	5 cc. Artificial Aggressin II intraperitoneally.	5 oesen suspension of "Pest Virulent."	Dead June 16, after 5 days.	Innumerable pest bacilli in smears from spleen.
1970	do	do	Dead June 18, after 7 days.	Numerous pest and post-mortem invading bacilli in smears from spleen.
1971	do	do	do	Numerous pest bacilli in smears from spleen.
1972	do	do	do	Do.
1973	do	do	Alive and well	
1974	do	do	Dead June 21, alter 9 days.	Pest bubo. Numerous pest bacilli in smears from spleen.
CONTROLS.				
2093		5 oesen suspension of "Pest Virulent."	Dead June 16, after 5 days.	Pest.
2094		do	Dead June 18, after 7 days.	Numerous pest bacilli in smears from spleen. Hæmorrhagic buboes.
2095		do	Dead June 16, after 5 days.	Pest.
2096		do	do	Do.
2097		do	Dead June 18, after 7 days.	Numerous pest bacilli in smears from spleen.

SERIES 36.—*Inoculations with artificial pest aggressin in guinea pigs.*

May 16: Sixty-seven 48-hour large agar slant cultures of "Pest Virulent" (from guinea pig number 1958, second generation) were suspended in 67 cubic centimeters distilled water. The suspension was then placed in a bottle, afterwards sealed and throughly shaken on an electrical machine for three days. On May 19 cultures were taken, these later showed numerous pest bacilli still alive. A 5 per cent solution of carbolic acid was added to the suspension sufficient to form a 0.5 per cent solution and it was then centrifuged for three hours at a velocity of 4,000 revolutions per minute. The centrifugated tubes were then removed and placed for two hours at a temperature between 45° and 46° C. The clear fluid above was then pipetted off and, after its sterility was proved, used on May 20 for the inoculations described in the table below. On July 16, two months later, the animals were all reinoculated in the following manner: 5 oesen of a suspension of "Pest Virulent" (one 48-hour culture from guinea pig number 2174, second transplant) in 5 cubic centimeters of bouillon were rubbed over a shaved area on the abdomen which was scarified with a scalpel.

SERIES 36—Continued.

It will be seen that these animals were inoculated in exactly the same manner as those used in the experiments described in Series 32 and 33. (See pp. 204, and 218.) The same twenty-five control guinea pigs, numbered 2098 to 2122, inoculated in the same manner, served for the present series as well. All of them died of acute pest infection.

Animal No.	Immunized May 20, with—	Infected July 16, with—	Result.	Autopsy and remarks.
2018	5 cc. Artificial Aggressin III.	5 oesen suspension "Pest Virulent" massaged over abdomen.	Dead July 22, after 6 days.	Moderate number of pest bacilli in smears from spleen.
2019	do	do	Dead July 23, after 7 days.	Innumerable pest bacilli in smears from spleen.
2020	do	do	do	Typical pest spleen.
2021	do	do	Dead July 21, after 5 days.	Few pest bacilli in smears from spleen.
2022	do	do	Dead July 23, after 7 days.	Typical pest spleen.
2023	do	do	Dead July 25, after 9 days.	Advanced bubo. Innumerable pest bacilli in smears from spleen.
2024	do	do	Dead July 23, after 7 days.	Numerous pest bacilli in smears from spleen.
2025	do	do	do	Few pest bacilli in smears from spleen.
2026	do	do	Dead July 25, after 9 days.	Typical pest spleen.
2027	3 cc. Artificial Aggressin III.	do	Dead July 23, after 7 days.	Innumerable pest bacilli in smears from spleen.
2028	2 cc. Artificial Aggressin III.	do	Dead July 25, after 8 days.	Numerous pest bacilli in smears from spleen.
2029	2½ cc. Artificial Aggressin III.	do	Dead July 23, after 7 days.	Do.

SERIES 42.—*Inoculations with artificial pest aggressin in guinea pigs.*

On July 16, sixty 48-hour agar cultures of the strain "Pest Virulent" (from guinea pigs numbered 2174 and 2175, second transplant) were suspended in 60 cubic centimeters distilled water, 1 cubic centimeter to each culture. The suspension was then placed on an electrical shaking machine for five days. The preparation of the artificial aggressin from this point on was then continued in exactly the same manner as described in Series 29 (p. 223). After it was centrifuged, cultures from the clear fluid showed the aggressin to be sterile.

On July 23 each guinea pig was inoculated subcutaneously with 5 cubic centimeters and again on August 1, each animal receiving a repetition of this dose. On August 24, about three weeks after the second inoculation the immunity of the animals was tested by massaging the abdomen with a portion of the spleens of guinea pigs numbered 2315 and 2316, just dead of acute plague infection. Two control animals were inoculated at the same time and in the same manner.

SERIES 42—Continued.

Animal No.	Immunized July 23 with—	Second immunization August 1.	Infected August 24.	Result.	Autopsy and remarks.
2197	5 cc. Artificial Aggressin IV.	5 cc. Artificial Aggressin IV.	Abdomen massaged with portions of the spleens of guinea pigs numbered 2315 and 2316 just dead of acute plague infection.	Dead Aug. 29, after 6 days.	Numerous pest bacilli in smears from spleen.
2198	do	do	do	Dead Sept. 2, after 9 days.	Subacute pest.
2199	do	do	do	Alive and well	
2200	do	do	do	do	
CONTROLS.					
2322			Abdomen massaged with portions of the spleens of guinea pigs numbered 2315 and 2316 just dead of acute plague infection.	Dead Aug. 29, after 5 days.	Pest bacilli in smears from spleen.
2323			do	Dead Aug. 31, after 7 days.	Do.

SERIES 34.—*Animals inoculated for the preparation of natural plague aggressin.*

Animal No.	Inoculated intraperitoneally with—	Result.	Abdominal exudate collected.
Rabbit 1966*	2 oesen of the heart's blood of guinea pig 1958 in 10 cc. bouillon on May 14.	Dead May 20, after 6 days.	2.75 cc. for Aggressin E.
Guinea pig 1964.	Portion of spleen of guinea pig No. 1957 shaken up in 5 cc. bouillon May 13.	Dead May 14, after 1½ days.	5 cc. for Aggressin A.
Guinea pig 1999.	½ oese "Pest Virulent" 3 days' culture, first transplant from guinea pig No. 1957 in 10 cc. bouillon May 17.	Dead May 19, after 2 days.	4 cc. for Aggressin C.
Guinea pig 2000.	do	Dead May 18, after 1 day.	4 cc. for Aggressin B.
Guinea pig 2001.	do	Dead May 19, after 2 days.	2 cc. for Aggressin C.
Guinea pig 2002.	do	Dead May 18, after 1 day.	4.5 cc. for Aggressin B.
Guinea pig 2003.	do	do	4 cc. for Aggressin B.
Guinea pig 2004.	do	do	3 cc. for Aggressin B.
Guinea pig 2007.	½ oese 6 days' culture "Pest Virulent" from guinea pig No. 1958 first transplant in 10 cc. bouillon.	Dead May 19, after 1 day.	3 cc. for Aggressin C.
Guinea pig 2008.	do	do	1.75 cc. for Aggressin D.
Guinea pig 2009.	do	Dead May 20, after 2 days.	4.5 cc. for Aggressin E.
Guinea pig 2010.	do	Dead May 19, after 1 day.	4.5 cc. for Aggressin D.
Guinea pig 2011.	do	Dead May 20, after 2 days.	2 cc., foul, containing many post-mortem bacilli. Not used.

*A number of rabbits were inoculated for the purpose of preparing natural aggressin. However, these animals were found in Manila to be unsatisfactory for this purpose owing to the rapid contamination of the fluid in the pleural or abdominal cavity due to post-mortem decomposition.

Series 34—Continued.

The abdominal exudates from these guinea pigs were collected on the day of the death of the animal and mixed with an equal volume of normal saline solution. Five per cent carbolic acid was then added in sufficient amount to form a 0.5 per cent solution. The mixtures were then heated for two hours at 44° to 45° C. and subsequently centrifuged for four hours at a velocity of 4,000 revolutions per minute. The clear fluid above was then pipetted and used in the inoculations described in Series 35. Its sterility was in each instance demonstrated.

Series 43.—*Guinea pigs inoculated for the preparation of natural plague aggressin.*

Animal No.	Inoculated intraperitoneally with—	Result.	Exudate obtained.
2201	1 oese "Pest Virulent" from guinea pig No. 2179 first transplant in 10 cc. bouillon July 23.	Dead July 26, after 3 days	5 cc. for Aggressin F.
2202	do	Dead July 25, after 2 days	3.5 cc. foul exudate obtained. Not used.
2203	do	Dead July 28, after 5 days	5 cc. for Aggressin G.
2210	½ oese 6-day culture of "Pest Virulent" guinea pig No. 2183 first transplant, in 5 cc. bouillon July 30.	Dead July 31, after 1 day	1 cc. for Aggressin H.
2211	do	Dead Aug. 1, after 2 days	3 cc. for Aggressin I.
2212	do	Dead July 31, after 1 day	1 cc. for Aggressin H.
2213	do	do	2 cc. for Aggressin H.
2214	do	do	1 cc. for Aggressin H.
2215	do	Dead Aug. 1, after 2 days	1 cc. for Aggressin I.
2216	do	Dead July 31, after 1 day	4 cc. for Aggressin H.
2217	do	do	Do.
2218	do	do	Do.
2219	do	Dead Aug. 1, after 2 days	2.5 cc. for Aggressin I.

The abdominal exudates from these guinea pigs were collected on the day of the death of the animal and mixed with an equal volume of normal saline solution. Five per cent carbolic acid was then added, sufficient to form a 0.5 per cent solution. The mixture was then heated for from one to two hours at a temperature of 45° to 46° C. and subsequently centrifuged for four to five hours at a velocity of 4,000 revolutions per minute. The clear fluid above was then pipetted off and used in the inoculations described in Series 44. The sterility of the aggressin was demonstrated in each instance.

Series 35.—*Inoculations with natural plague aggressin in guinea pigs.*

The animals were inoculated as below with natural aggressin, the preparation of which is described in Series 34. On July 16 they were all, with the exception of numbers 2053 and 2054 reinoculated by rubbing 5 oesen of a suspension of "Pest Virulent" (5 cubic centimeters bouillon to one 48-hour culture guinea pig numbered 2174, second transplant) over a shaved and lightly scarified area on the abdomen. These animals were inoculated in exactly the same manner as those given in Series 32 and 33 (see pp. 204 and 218), and the same twenty-five control guinea pigs numbered 2098 to 2122 were employed for the present series. Guinea pigs numbered 2053 and 2054 were inoculated June 18 by massaging a shaved area over the abdomen with a portion of the spleen of guinea pig numbered 2124 and scarifying the skin with a scalpel. Two control guinea pigs, numbered 2140 and 2141, were also inoculated in the same manner.

SERIES 35—Continued.

Animal No.	Immunized intraperitoneally with—	Reinoculated with—	Result.	Autopsy and remarks.
2012	5 cc. Natural Aggressin B, May 19.	5 oesen suspension of "Pest Virulent" over abdomen July 16.	Dead July 26, after 10 days.	Pest bubo. Innumerable pest bacilli in smears from spleen.
2013	do	do	Dead July 25, after 9 days.	Pest bubo. Very numerous pest bacilli in smears from spleen.
2014	4 cc. Natural Aggressin B, May 19.	do	Alive	
2015	do	do	do	
2016	2 cc. Natural Aggressin B, May 19.	do	do	
2017	do	do	do	
2030	3 cc. Natural Aggressin C, May 20.	do	Dead July 23, after 7 days.	Numerous pest bacilli in smears from spleen.
2031	do	do	do	Do.
2032	do	do	do	Pest. Typical bubo and spleen.
2033	3.5 cc. Natural Aggressin C, May 20.	do	Dead July 25, after 9 days.	Typical pest spleen. Fair number of pest bacilli in smears from spleen.
2034	2.5 cc. Natural Aggressin D, May 20.	do	Dead July 24, after 8 days.	Pest. Numerous pest bacilli in smears from spleen.
2035	do	do	Dead July 31, after 15 days.	Innumerable pest bacilli in smears from spleen.
2006	5 cc. Natural Aggressin A, May 15.	do	Dead July 26, after 6 days.	Numerous pest bacilli in smears from spleen.
2053	4.5 cc. Natural Aggressin E, May 21.	Abdomen massaged with portion of spleen of guinea pig No. 2124 on June 18.	Dead June 22, after 4 days.	Innumerable pest bacilli in smears from spleen.
2054	do	do	Dead June 25, after 7 days.	Comparatively few bacilli in smears from spleen.
CONTROLS.				
2140		Abdomen massaged with portion of spleen of guinea pig No. 2124 on June 18.	Dead June 25, after 7 days.	Innumerable pest bacilli in smears from spleen.
2141		do	Dead June 27, after 9 days.	Do.

And twenty-five other controls; all dead. (See Series 32 and 33, pp. 204 and 218.)

SERIES 44.—*Inoculations with natural plague aggressin in guinea pigs.*

The animals were inoculated intraperitoneally July 27 to August 3 as given below with natural aggressin, the preparation of which is described in Series 43 (p. 230). On August 28, about one month after the first inoculation, they were all reinoculated, 5 oesen of a suspension of "Pest Virulent" (one 48-hour slant culture from guinea pig numbered 2315 second transplant in 10 cubic centimeters bouillon) being rubbed over a freshly shaved area of the abdomen of the animal and the skin well scarified with a scalpel. Twenty-five control animals, numbered 2362 to 2386, were also inoculated in exactly the same manner and all died. The details of these autopsies were given in Series 41 (p. 210).

Animal No.	Inoculated intraperitoneally with—	Reinoculated August 28 with—	Result.	Autopsy and remarks.
2205	July 27, 4.5 cc. Natural Aggressin F.	5 oesen suspension "Pest Virulent" rubbed on abdomen.	Dead Sept. 4, after 7 days.	Numerous pest bacilli in smears from spleen.
2206	do	do	Alive and well	
2225	July 30, 4.5 cc. Natural Aggressin G.	do	Dead Sept. 15, after 18 days.	Typical bubo. No organisms found in smears from spleen.
2226	do	do	Alive and well	
2232	Aug. 1, 4 cc. Natural Aggressin H.	do	do	
2233	do	do	Dead Sept. 5, after 8 days.	Fair numbers of pest bacilli in smears from spleen.
2234	do	do	Dead Sept. 8, after 11 days.	Pest bubo. One or two abscesses in spleen.
2235	do	do	Dead Sept. 4, after 7 days.	Numerous pest bacilli in smears from spleen.
2236	do	do	Dead Sept. 7, after 10 days.	Innumerable pest bacilli in smears from spleen.
2237	do	do	Dead Sept. 4, after 7 days.	Do.
2269	Aug. 3, 4 cc. Natural Aggressin I.	do	Alive and well	
2270	do	do	Dead Sept. 9, after 12 days.	Innumerable pest bacilli in smears from spleen.

SERIES 54.—*Inoculations with Klein's method in guinea pigs.*

The buboes, lungs, spleen and liver containing necrotic foci were removed from three guinea pigs which had died of subacute plague infection. The organs were finely minced, spread out on glass in thin layers over sulphuric acid and dried at from 46° to 47° C. for four days, and at 45° C. for two days longer. The dried material was then rubbed up in a mortar with saline solution, the fluid portion being decanted off from the sediment which did not dissolve.

Each animal of the series received subcutaneously 2 cubic centimeters of this suspension, equal to about 25 milligrams of the powder. In two of the animals, numbered 2822 and 2823, large sloughs of the skin followed the inoculation. On November 26, about one month after the injection, the immunity of the animals was tested with the strain "Pest Virulent" in exactly the same manner as was

SERIES 54—Continued.

that of the animals comprising Series 46, 47, and 50 (pp. 211, 220 and 197). Twenty-five control animals, numbers 2870 to 2894, were also inoculated at the same time and in the same manner. All succumbed from acute plague infection. For the details of the protocols see Series 46.

Animal No.	Inoculated October subcutaneously with—	Reinoculated November 26 with—	Result.	Autopsy and remarks.
2821	2 cc. suspension of dried plague organs.	5 oesen suspension "Pest Virulent" rubbed over abdomen.	Dead Dec. 4, after 8 days	Typical bubo. Pest bacilli in spleen.
2822	do	do	do	Do.
2823	do	do	Dead Dec. 5, after 9 days	Do.
2824	do	do	Alive and well	
2825	do	do	do	
2826	do	do	Dead Dec. 3, after 7 days	Do.

SERIES 56.—*Inoculations by Klein's method in guinea pigs.*

The buboes, lungs, spleen and liver containing necrotic foci were removed from three guinea pigs, numbered 3017, 3018, and 3025 which had died of plague infection and were used for the manufacture of the prophylactic employed in these experiments. The prophylactic was prepared after the method of Klein as described in Series 54 (p. 232). Each animal received 2 cubic centimeters of the suspension. In three of the animals sloughs of the skin followed the inoculations. About one month after the inoculations the immunity of the animals was tested by rubbing 5 oesen of a suspension of "Pest Virulent" (one 48-hour agar slant culture from guinea pig number 3100, second transplant in 5 cubic centimeters bouillon) over a shaved area on the abdomen. Three control animals inoculated in the same manner and at the same time all succumbed to pest infection.

Animal No.	Inoculated subcutaneously with—	Reinoculated March 12 with—	Results.	Autopsy and remarks.
3035	2 cc. suspension dried plague aggressin.	5 oesen suspension "Pest Virulent" rubbed over abdomen.	Dead Mar. 16, after 4 days	Pest.
3036	do	do	Dead Mar. 20, after 8 days	Do.
3037	do	do	Alive and well	
3038	do	do	Dead Mar. 17, after 5 days	Pest.
3040	do	do	Dead Mar. 16, after 4 days	Do.
3046	do	do	Alive and well	
3047	do	do	Dead Mar. 18, after 6 days	Pest.
CONTROLS.				
3052		5 oesen suspension "Pest Virulent" rubbed over abdomen.	Dead Mar. 17, after 5 days	Pest.
3055		do	Dead Mar. 16, after 4 days	Do.
3056		do	Dead Mar. 16, after 4 days	Do.

DURATION OF IMMUNITY AFTER VACCINATION.

SERIES 55.—*Demonstrating duration of immunity in monkeys after vaccination.*

The animals comprising this series were vaccinated with the strains "Pest Avirulent" and "Maassen Alt" at different times, as may be seen from the table below. From nine to ten months after the vaccination they were all collected and on November 12 reinfected by the inoculation of ⅔ oese of the strain "Pest Virulent" (from guinea pig 2764, second transplant).

Animal No.	Vaccinated.	Reinoculated November 12.	Result.	Autopsy and remarks.
2842	Jan. 10	⅔ oese "Pest Virulent."	Dead Nov. 19, after 7 days	Pest bubo containing few organisms. No bacilli in smears from spleen.
2843	Jan. 12	do	Alive and well	
2844	Jan. 15	do	Dead Nov. 16, after 4 days	Numerous pest bacilli in smears from spleen.
2845	Jan. 16	do	Alive and well	
2846	Jan. 18	do	Dead Nov. 20, after 8 days	Few swollen pest bacilli in smears from spleen.
2847	Jan. 22	do	Dead Dec. 1, after 19 days	Chronic bubo containing few pest bacilli.
2848	Jan. 25	do	Dead Nov. 17, after 5 days	Numerous pest bacilli in smears from spleen.
2849	Jan. 27	do	Alive and well	
2850	Jan. 29	do	do	

VII. SUMMARY OF THE RESULTS OF THE ANIMAL EXPERIMENTS IN IMMUNIZATION BY THE DIFFERENT METHODS.

It will be seen from a study of the different series of experiments described on pages 187 to 234 that in many instances the results in immunization obtained by the same method of inoculation show considerable variation. This is particularly so in the series of monkeys inoculated in the early part of the work where the immunity of the animals in the different series was tested with varying amounts of the virulent pest organism. However, many difficulties were also encountered in the series in which the experiments were performed later. In the beginning of this article and in the records of some of the experiments, attention was called to the fact that the lethal dose for the species of monkeys employed varied considerably. In some instances over fifty times the lethal dose for one animal will not cause the death of another of about the same size. For this reason and because occasionally, in the earlier experiments, a control animal did not succumb to pest infection, a very large multiple of the lethal dose for the average monkey was finally employed; therefore, the percentage of these animals immunized was not as high as it might otherwise have been. It is undoubtedly for this reason that so low a percentage of the monkeys which had previously been inoculated with killed cultures of the pest organism subsequently resisted the infection. On the other hand, in my experiments with guinea pigs inoculated with killed cultures, the percentage of immunity obtained was as high as or higher than that frequently encountered by other authors.

Owing to this great individual variation in the susceptibility of different monkeys to pest infection, a fact discovered during the course of the inoculations, these animals did not prove to be as suitable as guinea pigs for the comparison of the value of the different methods. However, on the other hand, as has been mentioned above, the behavior of the monkey in relation to its resistance and immunity to pest infection probably much more closely resembles that encountered in man.

It is obvious that the series of inoculations which have been described are not selected as ideal ones. The entire number performed in relation to immunization by the different methods has been recorded in the order in which the experiments were carried on, the unfavorable as well as the favorable results being detailed. By no method was it possible by the employment of a single inoculation and a fixed dose, thoroughly to protect all of the animals of a series against infection. In fact a single vaccination of a number of animals of the series, even though the dose was so large as to cause the death of some of them, would not usually render thoroughly immune more than 75 to 80 per cent of the remainder. (See experiments with the strain "Maassen Alt.")

In spite of variations in the results of immunization sometimes obtained in the different series by the same method of inoculation, nevertheless, an examination of the experiments demonstrates conclusively and beyond any doubt the great value of vaccination against plague infection and its evident superiority to the other methods of immunization. No doubt of this fact is left after a study of the combined table of inoculations on page 238 where a comparison of the value of the different methods may be made at a glance.

In relation to the time which the immunity takes to develop and the time which it persists after vaccination, it may be stated that in three instances attempts were made to infect monkeys numbered 1581, 1593 and 1626 which had been vaccinated only six days previously. All of these animals remained alive and well. Nine monkeys, numbered 2842 to 2850 (see Series 55, p. 234), which had been vaccinated with the strain "Pest Avirulent" or "Maassen Alt" between nine and ten months previously were collected and their immunity tested by the inoculation of $\frac{2}{3}$ oese of the strain "Pest Virulent;" five of the animals succumbed and four (44 per cent) remained alive. In connection with these experiments it must be emphasized that the animals during the entire time between the vaccination and the infection (nine to ten months) had been in close captivity and were not in particularly good physical condition at the time they were infected. Moreover, it is obvious that in testing their immunity a much greater amount of plague bacilli ($\frac{2}{3}$ oese of the virulent strain) was employed than either man or animals would ever receive from a natural plague infection.

Prophylactic inoculations of natural aggressin appear to be next in value to *vaccination* as a means of immunization against pest. Inoculations with artificial plague aggressin did not, in my experiments, prove to be nearly as efficacious as those with natural aggressin. A much higher immunity was obtained with the latter prophylactic. However, as I have already pointed out elsewhere,[49] there was apparently no difference in the quality of the immunity obtained with the natural aggressin from that produced by the artificial product, and my subsequent experiments, as did my earlier ones, have only further confirmed the views of Wassermann and Citron[50] that the aggressins must be considered to be hypothetical substances and that, so far as their immunizing value is concerned, in these exudates we have to do mainly with the sustances extracted from the bacilli themselves. Evidently, in the case of the plague bacillus, the receptors of the organism in the so-called aggressin exudates of animals become liberated in a more efficient manner for immunization and probably exist in a less altered condition than they do in the aqueous suspension of the bacilli obtained by artificial means. Obviously, in natural plague aggressin no other immunizing substances are existent than those present in the prophylactic against pest recommended by Terni and Bandi. The two methods are practically identical as Bandi[51] has recently pointed out. However, it must be admitted that Bandi did not originally explain the principle of the action of his prophylactic, as we understand its action to-day after a study of the subjects of free receptors and of aggressins. The method of inoculation with natural plague aggressin is not likely to come into general use because of the great difficulties encountered in the preparation of the prophylactic. Moreover, in my experiments I have not obtained the satisfactory results with it which Hueppe and Kikuchi evidently anticipated. The method of *vaccination,* as already mentioned, gives a much greater degree of protection. Although Klein's method, according to the small number of experiments I have performed, gives about the same results in immunizing guinea pigs as are obtained by the inoculations with natural aggressin, yet the injection of the former substance as prepared in my experiments produced a much greater local reaction than the latter. Therefore, I could not ascertain that this method had any particular advantage over that in which inoculations of natural aggressin were employed.

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[49] *This Journal* (1906), 1, 512.

I performed no experiments with Lustig's plague prophylactic, partly because of the poor results which Kolle and Otto[52] encountered in their experiments and partly because the results which have been obtained in this laboratory by the use of this method in extracting the immunizing substances from the cholera spirillum have not been encouraging for its further use; other methods of extraction of these substances from the bacilli having in my opinion given more satisfactory results.

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[50] *Deutsche Med. Wchnschr.* (1905), **31**, 1101.

[51] *Centrbl. f. Bakteriol.* (1906), **42**, 448.

[52] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1903), **45**, 517.

Combined table comparing efficiency of different methods of immunization.

Kind of animal and series No.	Number inoculated.	Number dead before immunity tested.	Immunized.	
			Number.	Percentage.
KILLED PEST CULTURES.				
Monkeys (bouillon cultures):				
Series 5	8	------	3	37
Series 49	9	------	2	22
Monkeys (agar cultures):				
Series 9	3	------	------	------
Series 23*	[18]	[3]	[11]	[72]
Series 25	20	3	4	23
Series 48	15	1	4	28
Total	*55	4	13	25
Guinea pigs (killed agar cultures):				
Series 50 (total)	15	------	4	26
"PEST AVIRULENT."				
Monkeys:				
Series 4	6	------	5	83
Series 11	5	------	1	20
Series 12	8	------	4	50
Series 18	10	------	5	50
Series 51	15	------	8	53
Total	44	------	23	52
Guinea pigs:				
Series 32	11	5	5	83
Series 37	9	2	6	85
Series 39	15	------	8	53
Series 41	21	------	15	71
Series 46	15	------	12	80
Total	71	7	46	72
"MAASSEN ALT."				
Monkeys:				
Series 17	4	------	3	75
Series 21	12	------	8	66⅔
Series 24	18	4	13	92
Series 52	15	1	7	50
Total	49	5	31	70
"MAASSEN ALT"—Continued.				
Guinea pigs:				
Series 33	10	3	7	100
Series 38	7	1	4	66⅔
Series 40	15	1	11	78
Series 47	15	1	14	100
Total	47	6	36	88
EXTRACTS OF PLAGUE BACILLUS (FREE RECEPTORS).				
Monkeys:				
Series 7	4	------	1	25
Series 26	5	------	1	20
Total	9	------	2	22
ARTIFICIAL AGGRESSIN.				
Monkeys:				
Series 29 (total)	32	------	4	12½
Guinea pigs:				
Series 29	2	------	0	0
Series 30	2	------	0	0
Series 31	6	------	1	16⅔
Series 36	12	------	0	0
Series 42	4	------	2	50
Total	26	------	3	11
NATURAL AGGRESSIN.				
Guinea pigs:				
Series 35	15	------	4	26
Series 44	12	------	4	33⅓
Total	27	------	8	30
KLEIN'S METHOD.				
Guinea pigs:				
Series 54	6	------	2	33⅓
Series 56	7	------	2	28
Total	13	------	4	30

*Series 23 has not been considered in the total. See text.

VIII. THE FORMATION OF AGGLUTININS IN PLAGUE IMMUNE SERUM.

After having concluded from the experiments recited above that true plague vaccination (inoculation with living, attenuated cultures) produced the highest immunity, inoculations were made in human beings with the strain "Pest Avirulent." In order to ascertain if any evidences of immunity could be demonstrated in the inoculated, their blood was tested for the presence of agglutinins, anti-infectious substances and opsonins. This led me to investigate in detail whether, and if so, to what extent, these same anti-bodies existed in the blood sera of animals which had been immunized against plague infection.

AGGLUTINATION OF THE PEST BACILLUS.

Wyssokowitz and Zabolotny[53] and the German Plague Commission called attention to the fact that the blood serum of persons who had suffered an attack of plague sometimes showed a weak agglutinating action against the pest bacillus. The German Plague Commission further emphasized that under these circumstances the reaction was not always present and that its occurrence bore no relation to the severity of the disease, since in the most severe case which they examined it was absent, while in a mild case of plague, the strongest positive reaction was obtained. They studied the sera of two persons who about three weeks before had been vaccinated against pest by Haffkine, but found no trace of an agglutinating reaction. They also examined four different pest immune sera produced in horses and in three found practically no agglutinative action or only traces of it; but one of the sera showed a suggestion of a reaction above the dilution of 1 to 20. A goat which received four injections of killed cultures of the pest bacillus, six cultures being finally injected, was killed about one week after the final inoculation. Its blood serum then showed a strong agglutinative action in a dilution of 1 to 30. The commission conclude that in plague infection the agglutination is not at all parallel with the protective and immunizing power of the serum. Pest sera which showed a strong agglutinating reaction, in the animal body proved to be entirely inactive against pest infection and *vice versa.*

Zabolotny[54] also found that the agglutinative action of the blood serum of those who had suffered with pest was very inconstant. In some cases which had been inoculated with Haffkine's prophylactic, he found a weak agglutinative power which, however, was not so marked as in cases which had recovered from a natural attack of the disease.

Vagedes[55] investigated the agglutinative action of the sera of thirteen persons who had suffered with pest in Oporto. In only two cases was a very weak, positive result obtained. The blood serum was collected from one to four months after the beginning of the illness.

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[53] *Ann. d. l'inst. Pasteur* (1897), **11**, 662.

[54] *Arch. d. Sci. Biologiques* (1901), **8**, 85.

[55] *Klin. Jahrbch.* (1900), **7**, 537, and *Arb. a. d. k. Gsndhtsamte Berl.* (1900), **17**, 181.

Uriate[56] in a study of epidemics of pest in Paraguay, Rosario and Buenos Ayres, found that the sero-agglutination of the pest organism occurred in cases of plague only late in the course of the disease and then very irregularly. It failed actually in more than 300 cases which, from a bacteriological and clinical standpoint, were certainly those of pest.

On the other hand Paltauf,[57] Markl[58] and Kolle and Martini[59] showed that a specific agglutination of the organism occurred in various dilutions with pest immune sera produced in horses and other animals by repeated injections of the plague bacillus. Paltauf found that in horses immunized by repeated inoculations a reaction occurred in dilutions of 1 to 20. A distinct agglutination occurred within one hour in dilutions of 1 to 100 with the sera with which Markl worked. Kolle and Martini with less virulent cultures of the pest bacillus obtained a reaction within one hour in dilutions of 1 to 1,000 to 1 to 6,000. Kolle and Otto[60] in the study of two pest immune sera found that agglutination occurred within fifteen minutes in as high dilutions as 1 to 400. Klein[61] also reported that the blood of vaccinated guinea pigs showed an agglutinative value in dilutions of 1 to 20 or 1 to 30, the reaction occurring in fifteen minutes.

The German Plague Commission and Kossel and Overbeck[62] emphasized the difficulty or impossibility of securing a suspension of the freshly grown pest bacillus which microscopically was free from clumps of the organism, and therefore they recommended that the test should be made macroscopically in the test tube and the reaction observed with the assistance of a hand lens. The time recommended for the observation of the test was from one-half to one hour, the suspension being placed in the incubator at 37° C. for this period. However, Kolle and Martini recommended that the reaction be noted after five minutes, since in their experience spontaneous precipitation of the bacteria sometimes occurred after one hour.

Klein[63] pointed out that cultures of the pest bacillus on agar possess a sticky, viscid growth due to the production of a gelatinous interstitial substance which is insoluble in bouillon. He therefore recommended physiological salt solution as a medium for preparing the suspension. He also found that the addition of bouillon to a saline suspension of the pest organism caused clumping of the bacteria.

Gauthier and Raybaud[64] found that precipitation of the bacteria also frequently occurred with plague cultures grown on gelatine and suspended in saline solution and that after two hours a spontaneous, flocculent sedimentation which resembled true agglutination was apt to form.

In my studies of the agglutination of the pest bacillus, I have frequently found the same difficulty in securing complete suspensions of the organisms owing to their sticky, viscid growth, even when saline solution has been used. The method employed in making these suspensions has been as follows:

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[56] Mitt. a. d. sektion f. Hyg. d. Kongress des Ass. francaise pour l'avancement des sci. tenu Grenoble 1904, *Arch. f. Schiffs u. Trop. Hyg.* (1905), **9**, 89.

[57] *Wien. Klin. Wchnsch.* (1897), **10**, 537.

[58] *Centrbl. f. Bakteriol.* (1901), **29**, 810.

[59] *Deutsche med. Wchnsch.* (1902), **28**, 46.

[60] *Ztschr. f. Hyg. u. Infectionskrankh.* Leipz. (1902), **40**, 595.

[61] *Arb. a. d. k. Gsndhtsamte,* Berl. (1902), **18**, 114.

[62] *Lancet* (1901), **1**, 456, 1535.

[63] *Ibid.*

[64] *Compt. rend. Soc. de biol.*, Par. (1904), **56**, 391.

> One cubic centimeter of an 0.85 per cent saline solution has been placed in a small test tube and 1 oese of a 24-or 48-hour agar culture of the organism has been introduced into the tube, thoroughly rubbed up against the walls by means of the oese and the bacteria gradually moistened with the saline solution. While the suspensions obtained with some pest cultures in this manner are satisfactory, a complete suspension of the organism can not be produced with others and it is necessary to combine the suspensions and either filter or allow them to stand until the larger clumps of bacteria have settled to the bottom of the tube, when the supernatant fluid may be drawn off with a pipette.

However, while satisfactory suspensions are apparently obtained by these methods, the greatest difficulty is experienced with certain cultures of this organism in keeping the bacteria in suspension for a longer period than, at most, one or two hours. After this time the organisms begin to precipitate and sometimes at the end of two or three hours the bacteria may have settled almost entirely to the bottom of the tube, the supernatant fluid above appearing almost clear. This phenomenon is particularly marked with the much attenuated plague cultures.

I have followed the suggestion of Shibayama[65] of growing the bacillus in the ice box at a temperature of from 5° to 8° C., with the object of obtaining a less sticky and viscid growth of the pest bacillus and thus securing a more satisfactory suspension of the organism in saline solution. However, while I have found that with cultures which have grown for a number of generations at such a temperature a much less viscid growth apparently is obtained and one which may easily be suspended in the saline solution, on the other hand the bacteria so grown become spontaneously precipitated from the saline suspensions in a much shorter time than do the organisms of the same strain grown at a temperature of 30° C. I have also found that the addition of normal serum to suspensions of the pest bacilli in saline solution sometimes retards and sometimes increases the tendency of the bacteria to settle out of the fluid. For these reasons pseudo-reactions are not infrequently encountered in performing agglutination tests with the plague bacillus, and great care is sometimes necessary to distinguish such reactions from those of true agglutination. Therefore, it is advisable to perform in duplicate all tests for agglutination with the different dilutions of the serum and at the same time to carry on a parallel series of experiments in the same dilutions with a normal serum from an animal of the same species and, moreover, to repeat all the tests with another transplant of the same culture upon the following day. Only by taking these precautions is it possible to distinguish certain pseudo-agglutinations of the pest bacillus from true ones. When testing the agglutinative reaction of supposed plague immune sera, I have occasionally encountered pseudo-agglutinative reactions in the control tubes containing normal serum and suspensions of plague bacilli, which could not be distinguished from those of true agglutination. In the tubes in some instances were seen typical, flocculent precipitates, visible to the naked eye and which settled to the bottom, leaving the overlying liquid clear. Such reactions occurred at the same time in the same dilutions of the immune serum which was being tested, but not in the test tubes containing the suspensions of bacilli alone, and hence if the control tubes with normal serum had not been prepared, the reactions unquestionably would have been considered as true agglutinative ones, which they certainly were not. It is true that the precipitation obtained in these instances disappeared to a greater or less extent on shaking the tube and it then re-formed slowly, but notwithstanding statements in the literature to the contrary, the same phenomenon (disappearance of the precipitation on shaking) occurs in the true agglutination not only of plague but also of cholera, and certain other microorganisms.

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[65] *Centrbl. f. Bakteriol. Orig.* (1905), **38**, 482.

From what has been said above it is evident that the time limit for the completion of the agglutinative reaction of the plague bacillus is very important. Most observers have limited the time of the reaction to within two hours. However, Shibayama,[66] who concluded that 72-hour cultures of the plague bacillus, cultivated in the ice box, are agglutinated in much higher dilutions than are the same strains grown at 32° to 37° C., drew his conclusions from an examination of the reaction after twenty-four hours. In this connection I here shall record some recent experiments performed with two sera obtained from monkeys which had been immunized against plague and with one serum from, a normal monkey. The agglutinative values of these three sera were tested in dilutions of 1 to 10 and 1 to 20, first with 72-hour cultures of a virulent pest strain which had been cultivated for six generations in the ice box at a temperature of from 5° to 10° C. and second with 48-hour cultures of the same strain cultivated for the same number of generations at 30° C. The readings of the reactions were made after one and one-half hours and were all found to be negative, no traces of agglutination appearing in any of the tubes. After sixteen hours the tubes were all examined again. At this time in those containing the suspensions which were made with the bacteria cultivated at 5° to 10° C. there was almost complete precipitation of the organisms, the overlying liquid being clear in all, including the control tubes with normal serum and those containing suspensions of the bacteria in saline solution with no serum, as well as the tubes with the immune sera. In the tubes which contained the serum and suspensions of the organisms cultivated at 30° C. there was almost no precipitation of the bacteria, the fluid still being cloudy. My experience has led me to conclude that the time limit of the agglutinative reaction with the plague bacillus should be placed at certainly not over one or two hours at 37° C. at the most, since after this time the results may be very inaccurate and confusing owing to the tendency of the bacteria to become spontaneously precipitated.

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[66] *Loc. cit.*

The testing of serum supposed to contain relatively small amounts of plague agglutinins has been very unsatisfactory where it was necessary to employ concentrated mixtures of the serum, for example in dilutions of 0.5 or 0.25. As already mentioned, the density and viscosity of the serum in these dilutions evidently somewhat retards the agglutination of the pest organism, as was evident from the fact that the reaction occurred in some of the experiments in the higher but not in the lower dilutions. This phenomenon of retardation can not be explained on the assumption of the presence of agglutinoid, since it was noted in several instances with perfectly fresh sera. It may sometimes be observed in fresh, specific immune sera with other bacteria and must be ascribed to physical causes, the discussion of the nature of which will not be entered into here.

I have concluded from a large number of experiments that the agglutinins are formed slowly and only in very small amounts in animals which are being immunized against pest infection and that they only occur in demonstrable quantities in those which have been very highly immunized. At most, only very minute traces of these substances are encountered after single inoculations of either the killed or the living organisms, no matter how large the dose which is employed. I am convinced that in my earlier experiments with plague agglutination I sometimes mistook pseudo-agglutinations of the pest bacillus for true ones, and from a study of the literature it seems to me very likely that other observers have also erred in this respect. A study which I have made of the blood sera of guinea pigs which have been vaccinated against pest infection and which have later shown themselves to be immune to lethal and multiple lethal doses of the pest bacillus has demonstrated that practically no traces of agglutinins exist in such sera. The same may be said of the sera of other animals immunized in a similar manner. Monkeys which have first been vaccinated with attenuated pest cultures or inoculated with killed cultures, and which have afterwards been shown to be immune to pest infection by the injection in increasing doses of from ½ oese to nearly 1 entire agar slant culture of the living virulent strain, still have developed in their sera practically no traces of agglutinins. Indeed it is very difficult to immunize monkeys to such a high degree that the blood of the animals shows the presence of plague agglutinins. In a series comprising twelve monkeys (numbers 1232, 1233, 1239, 1240, 1247, 1286, 1287, 1300, 1301, 1302, 1357, 1358), in which an attempt was made gradually to immunize these animals with the strain "Pest Virulent" up to such a degree that these anti-bodies would be demonstrable in their blood, only two were able to survive the inoculations when the immunization had reached the point in which the agglutinins could be detected even in small amounts. (Numbers 1300 and 1357. See agglutination experiments, p. 246.) The remaining ten animals succumbed either to pest infection or intoxication as a result of the injections, before agglutinins could be shown to exist in their blood. Moreover, notwithstanding the fact that from ¼ to ½ oese of this strain "Pest Virulent" represented a certain lethal dose for normal animals of this species and although these monkeys were immunized to such an extent that in a number of instances they were able to resist and survive the inoculations of such large amounts as 6 to 8 oesen of this organism (over twelve times the maximum fatal dose), agglutinins were still not present in sufficient quantities to be demonstrable in their blood.

Only small quantities of agglutinins could be detected in the examination of several pest immune sera which were prepared from horses and which were known to possess, in two instances at least, considerable protective (anti-infectious) power.

A horse which was being immunized against pest and which had acquired a sufficient immunity to withstand the injection of nearly 10 agar cultures of a virulent pest strain, gave a serum which at this time showed an agglutinative reaction in dilutions of 1 to 10, but none above this strength. However, at the same time 1 cubic centimeter of this serum protected from fatal pest infection about 60 per cent of the white rats inoculated. (See Series 45), p. 284. A pest immune serum obtained from Asia, which protected about 72 per cent of the inoculated rats against fatal pest infection in doses of from 1 to 2 cubic centimeters (see Series 8, 10, and 20, pp. 274, and 281), when carefully tested with the virulent pest strain showed no agglutinative reaction after three hours. However, it must be stated that at the time its anti-infectious and agglutinative reactions were tested this serum had been bottled for about a year. A second pest serum purchased from Asia which showed a somewhat lower protective power (see Series 27, p. 283) also revealed no agglutination when tested with the strain "Pest Avirulent." This serum had been bottled about nine months. Moreover, a laboratory immune serum which possessed a higher anti-infectious power and which protected about 90 per cent of the inoculated white rats against plague infection (see Series 53, p. 286) in doses of 1 cubic centimeter also showed almost no agglutinative reaction against the virulent strain giving only a weak reaction in a dilution of 1 to 10.

The following selected experiments justify the preceding remarks upon agglutination. Experiments performed with a number of animals less highly immunized and in which the blood also showed no agglutination will not be given. The reactions were all performed with the virulent strain, excepting in a few instances when it is so stated, and by the macroscopic method. One oese of the bacteria was suspended in 1 cubic centimeter, 0.85 per cent saline solution and 1 cubic centimeter of the serum to be tested, diluted with saline solution, was added to the suspension. It is perhaps unnecessary to state that the microscopic method of observing agglutination with the plague bacillus is entirely untrustworthy.

DETAILS OF THE EXPERIMENTS.

Monkey number 1231.—Vaccinated October 10 with 1 culture "Pest Avirulent." Reinoculated October 20 by thrusting beneath the skin a 5 cubic centimeter syringe needle which had been dipped in a suspension of "Pest Virulent." On October 28, 1 oese "Pest Virulent" was inoculated subcutaneously; on November 16 the animal was killed, and the agglutination of the serum tested in dilutions 1: 2 and 1: 4; two controls of the reactions (of the bacterial suspension without serum) were performed. All were negative after one and four hours.

Rabbit number 1960.—May 11: One 48-hour slant culture of "Pest Avirulent" in 1 cubic centimeter of bouillon was injected intravenously. The agglutination was tested twelve days later in dilutions of 1: 20, 1: 40, 1: 80, 1: 160, 1: 320, two controls of the reaction (of the bacterial suspension without serum) were prepared. All the reactions were negative after one and three-fourths hours. After sixteen hours, pseudo-reactions in dilutions of 1: 20 to 1: 60 were observed, the bacteria being precipitated and the suspensions appearing clear; the two control reactions were negative. The serum was again tested on the following day in dilutions of 1: 40, 1: 80, 1: 160, with two controls. The reactions were all negative after one hour. After sixteen hours, pseudo-reactions in the dilution of 1: 40 were observed. Three days later the serum was again tested in dilutions of 1: 5 and 1: 10 and two controls (without serum). The results were negative after one and one-half hours. After sixteen hours pseudo-reactions in dilutions of 1: 5 and 1: 10 were observed; both controls were negative.

Normal rabbit's serum was tested on this same date in dilutions of 1: 5 and 1: 10, with two controls of the bacterial suspension. All were negative after one and one-half hours. After sixteen hours pseudo-reactions in dilutions of 1: 5 and 1: 10 were observed; both controls were negative.

Rabbit number 1961.—May 12: One 48-hour culture of "Pest Avirulent," suspended in 1 cubic centimeter of bouillon, was injected intravenously. Twelve days later the animal was killed and the agglutination tested in dilutions of 1: 20, 1: 40, 1: 80, 1: 160, 1: 320. Three controls of the reactions without serum and 1 control with normal rabbit serum in a dilution of 1: 30 were performed. After one hour pseudo-agglutination was observed in the dilutions of 1: 20 and 1: 40. The remaining tubes were all negative; after sixteen hours pseudo-reactions took place in dilutions of from 1: 20 to 1: 80. The same serum tested the following day in dilutions of 1: 2 gave no agglutination after one and one-half hours.

Rabbit number 1963.—Inoculated intravenously with 5 cubic centimeters of Haffkine's prophylactic. Five days after the inoculation the animal was killed and the agglutination tested in dilutions of 1: 2 and 1: 5, with two controls. The results were negative after two and sixteen hours.

Rabbit number 1965.—Inoculated with 5 cubic centimeters of Haffkine's prophylactic intravenously. Ten days later the animal was killed and the agglutination tested in dilutions of 1: 20, 1: 40, 1: 80, 1: 160, 1: 320; three controls of the bacterial suspension without serum and two controls with normal serum in dilutions of 1: 10 and 1: 40 were prepared. After one hour all reactions were negative; after sixteen hours precipitation of the bacteria occurred in dilutions of 1: 20 and 1: 40 and in the normal serum in dilutions of 1: 10. The same serum tested the following day; in dilutions of 1: 2, gave a reaction after one and one-half hours.

Rabbit number 1967.—Two cubic centimeters of the autolyzed product obtained from 20 milligrams of the strain "Pest Virulent" were injected intravenously; the animal was killed ten days later and the agglutination in dilution of 1: 2 tested. The reaction was negative after one and one-half hours.

Rabbit number 1968.—Inoculated in the same manner as number 1967 and with the same results.

Monkey number 1278.—Inoculated subcutaneously October 19 with 1 agar culture of "Pest Avirulent;" on October 30 inoculated subcutaneously with 2 oesen of "Pest Virulent." On November 17 the animal was killed and the agglutination tested in dilutions of 1: 2, 1: 4, with two controls without serum. The reactions were all negative after four hours.

Monkey number 1285.—Inoculated October 20 by thrusting beneath the skin a 5 cubic centimeter syringe needle dipped in a suspension of the strain "Pest Virulent;" on October 25 the animal was reinoculated with 2 oesen of "Pest Virulent;" on November 17 it was killed and the agglutination tested in dilutions of 1: 2 and 1: 4, with two controls of the bacterial suspension without serum. All reactions were negative after two hours.

Monkey number 1286.—October 20: The skin over the abdomen was scarified and a suspension of "Pest Virulent" rubbed over this area. On October 25, 1 oese of "Pest Virulent" was injected subcutaneously; on December 2, ⅖ of an agar slant of "Pest Virulent" was given subcutaneously; on December 14, ⅗ of an agar slant of "Pest Virulent" was injected subcutaneously; on December 16 the animal died. The serum was tested on November 17 in dilutions of 1: 2 and 1: 4. Both were negative after two hours.

Monkey number 1287.—Inoculated exactly as monkey number 1286 up to December 14; the animal died on December 15, the day after the last inoculation. The serum was tested on the following day in dilutions of 1: 2 and 1: 4; the result was negative after two hours.

Monkey number 1300.—October 25, two agar slant cultures of "Pest Avirulent" injected subcutaneously; November 10, ½ oese "Pest Virulent;" on December 2, 2 oesen of "Pest Virulent" were given; on December 14, 4 oesen "Pest Virulent;" on February 15, one 48-hour agar slant of "Pest Virulent" and on February 27, two 48-hour agar slants "Pest Virulent" were injected. March 10 the agglutination was tested in dilutions of 1: 2 and 1: 10. After two hours there was positive agglutination in dilutions of 1: 2, the fluid being almost entirely clear. A slight precipitation of the bacteria was observed in the dilution of 1: 10.

Monkey number 1357.—November 2, infected with a 10 cubic centimeter syringe needle dipped in a suspension of "Pest Virulent;" on December 2, ⅕ of an agar slant of "Pest Virulent" was inoculated subcutaneously; on December 14, ⅗ of an agar slant subcutaneously; on January 10, 1 agar slant of "Pest Virulent;" on February 1, 1½ agar slants of "Pest Virulent;" on February 27, 2 agar slants of "Pest Virulent;" on March 15, 3 agar slants of "Pest Virulent." The animal died on March 17. The agglutination in the dilutions of 1: 5 was positive, the fluid being clear after one hour; in the dilution of 1: 10 there was a positive weak reaction after four hours.

Monkey number 2666.—Inoculated October 27. One 48-hour culture of *killed* "Pest Virulent" being given subcutaneously; on November 28, reinoculated with

⅔ oese "Pest Virulent;" on December 28 with 9 cultures of killed "Pest Virulent," subcutaneously. On January 10, the animal was killed. The agglutination tested in dilutions of 1: 5, 1: 10 and 1: 20 were all negative after two hours.

Monkey number 2720.—October 29, one agar culture "Pest Avirulent" injected subcutaneously; on November 28, ⅔ oese "Pest Virulent" given subcutaneously; on December 29, 1 oese of "Pest Virulent" given subcutaneously; on February 7 the animal was bled and the serum tested for agglutination. The serum of monkey number 2666 (see above) and of monkey number 3053 (normal serum) were also both tested on this date with 72-hour cultures of "Pest Virulent" grown in the ice box for six generations and with cultures grown for the same length of time and for the same number of generations at 30° C.

The results obtained are as follows:

TABLE No. I.—*Serum of immune monkey number 2666—Agglutination tests with cultures grown for six generations in ice box and at 30° C.*

[The term "control" throughout the experiments signifies a suspension of the bacteria in saline solution without the addition of serum.]

<table>
<tr><th colspan="3">GROWN IN ICE BOX.</th></tr>
<tr><th>Dilutions.</th><th>After 1½ hours.</th><th>After 16 hours.</th></tr>
<tr><td>1:10</td><td>Negative</td><td>Heavy precipitate. Fluid clear.</td></tr>
<tr><td>1:20</td><td>do</td><td>Do.</td></tr>
<tr><td>Control.</td><td>do</td><td>Do.</td></tr>
<tr><td>Control.</td><td>do</td><td>Do.</td></tr>
<tr><th colspan="3">GROWN AT 30° C.</th></tr>
<tr><td>1:10</td><td>Negative</td><td>Negative. Fluid not clear. Practically no precipitate.</td></tr>
<tr><td>1:20</td><td>do</td><td>Do.</td></tr>
<tr><td>Control.</td><td>do</td><td>Do.</td></tr>
<tr><td>Control.</td><td>do</td><td>Do.</td></tr>
</table>

TABLE No. II.—*Serum of immune monkey number 2720—Agglutination tests with cultures grown for six generations in ice box and at 30° C.*

<table>
<tr><th colspan="3">GROWN IN ICE BOX.</th></tr>
<tr><th>Dilutions.</th><th>After 1½ hours.</th><th>After 16 hours.</th></tr>
<tr><td>1:10</td><td>Negative</td><td>Heavy precipitate. Fluid almost clear.</td></tr>
<tr><td>1:20</td><td>do</td><td>Heavy precipitate. Fluid clear.</td></tr>
<tr><td>Control.</td><td>do</td><td>Do.</td></tr>
<tr><td>Control.</td><td>do</td><td>Do.</td></tr>
<tr><th colspan="3">GROWN AT 30° C.</th></tr>
<tr><td>1:10</td><td>Negative</td><td>Slight precipitate. Fluid not clear.</td></tr>
<tr><td>1:20</td><td>do</td><td>Negative.</td></tr>
<tr><td>Control.</td><td>do</td><td>Do.</td></tr>
<tr><td>Control.</td><td>do</td><td>Do.</td></tr>
</table>

TABLE NO. III.—*Normal serum of monkey number 3053—Agglutination tests with cultures grown for six generations in ice box and at 30° C.*

GROWN IN ICE BOX.		
Dilutions.	After 1½ hours.	After 16 hours.
1:10	Negative	Heavy precipitate. Fluid almost clear.
1:20	do	Do.
Control.	do	Do.
Control.	do	Do.
GROWN AT 30° C.		
1:10	Negative	Slight precipitate. Fluid not clear.
1:20	do	Do.
Control.	do	Do.
Control.	do	Do.

Guinea pig number 3031.—Inoculated subcutaneously January 29 with one-half 24-hour culture of "Pest Virulent" partially attenuated. The animal died February 11, thirteen days after its inoculation, of advanced pest pneumonia and pest bubo. Agglutinative values of the serum of this animal and of the serum of a normal guinea pig (number 3053) were tested in dilutions of 1: 5 and 1: 10; the reactions were negative after two hours. After three hours, a moderate precipitation occurred in all of the tubes; quite as marked in those without serum as in the tubes with normal serum and in those of the pest guinea pig serum.

Guinea pig number 2636.—October 26, one culture of "Pest Avirulent" given subcutaneously.

Guinea pig number 2648.—October 26, one culture "Pest Maassen Alt" given subcutaneously. On November 26 both animals were reinoculated *cutaneously* with 5 oesen of a suspension of "Pest Virulent;" on January 17 they were reinoculated cutaneously with a portion of the spleen of a guinea pig (number 2993) which had just died of pest; the abdomen being deeply scarified. On February 7, both animals were killed and the agglutination of their sera, together with normal guinea pig serum (number 3053), tested in dilutions of 1: 2 and 1: 4. The reactions were all negative after one hour; after sixteen hours a precipitation of the bacteria in nearly all of the tubes occurred.

Pest immune horse serum Number I purchased from Asia. This serum in doses of 1 to 2 cubic centimeters protected against fatal pest infection about 72 per cent of the rats inoculated. (See Series 8, 10, and 20, pp. 274, 275 and 281.) The agglutination was tested with the strain "Pest Virulent" (second transplant) in dilutions of 1: 2, 1: 5, 1: 10, 1: 20; controls with normal horse serum in dilutions of 1: 2, 1: 5, 1: 10, and two controls with saline solution without serum were also prepared. After three hours all the reactions were negative; after sixteen hours sedimentation occurred in the majority of the tubes. In none of the tubes was the sediment more marked than it was in one of the control tubes of the bacteria without serum.

Pest immune serum Number II (obtained from horse), its anti-infectious power (see Series 27) being somewhat lower than that of Serum Number I. The agglutinative value was tested with three strains of pest bacilli of different virulence; at the same time a normal monkey serum was tested for control purposes. The results obtained are as follows:

TABLE NO. IV.—*Foreign Serum Number II and normal monkey serum—Agglutination tests January 17.*

Dilutions.		After 1 hour.	After 1½ hours.	After 2 hours.	After 21 hours.
FOREIGN SERUM NUMBER II.					
Virulent strain.	1:2	Negative	Slight precipitate.	Distinct precipitate.	Marked precipitate. Fluid not entirely clear.
	1:10	do	do	do	Very heavy precipitate. Fluid cleared.
	Control.	do	Negative	Negative	Negative.
Avirulent strain.	1:2	do	Positive		Heavy precipitate. Fluid cleared.
	1:10	Positive	do		Do.
	Control.	Negative	do		Do.
NORMAL MONKEY SERUM.					
Virulent strain.	1:2	Negative	Negative	Negative	Negative.
	1:10	do	do	Bacteria beginning to sediment.	Heavy precipitate. Fluid not entirely clear.
	Control.	do	do	Negative	Negative.
Avirulent strain.	1:2		Positive		Moderate precipitate. Fluid not clear.
	1:10		do		Heavy precipitate. Fluid entirely cleared.
	Control.		do		Do.

From the above experiments we can conclude only that the precipitate of the bacteria is coarser grained and that it occurs somewhat earlier in the immune serum than in the normal monkey serum.

TABLE NO. V.—*Foreign Serum Number II and immune monkey serum—Agglutination tests January 18.*

Dilutions.		After 1½ hours.	After 2 hours.	After 5 hours.
FOREIGN SERUM NUMBER II.				
Maassen strain.	1:2	Negative	Negative	Negative.
	1:10	do	do	Do.
	1:20	do	Sediment beginning to form	Positive weak.
	Control.	do	Negative	Negative.
	Control.	do	do	Do.
Virulent strain.	1:10	do	do	Do.
	Control.	do	do	Do.
IMMUNE MONKEY SERUM.				
Maassen strain.	1:2	Negative	Negative	Negative.
	1:10	do	Positive*	Positive.
	1:20	do	do.*	Do.
	Control.	do	Negative	Negative.
	Control.	do	do	Do.
Virulent strain.	1:10	do	do	After 5 hours.*
	Control.	do	do	Negative.

*A sedimentation is occurring and particles are collecting in the fluid, strongly simulating agglutination.

Pest immune serum Number III from a horse in the process of immunization by Dr. Ruediger. The last injection before bleeding was with nearly 10 agar cultures of "Pest Virulent" intravenously. The serum was drawn and tested for agglutination fifteen days after this injection. A horse's normal serum was also tested at the same time. The results are as follows:

TABLE No. VI.—*Immune serum Number III and normal serum (for control)—January 23, agglutination with strain "Pest Virulent" second transplant, 24-hour cultures.*

IMMUNE SERUM NUMBER III.			
Dilution.	After 1 hour.	After 4 hours.	After 20 hours.
1:2	Negative	Positive	Positive.
1:10	Positive	do	Do.
NORMAL SERUM (FOR CONTROL).			
1:2	Negative	Negative	Negative.
1:10	do	do	Very slight sediment.
Control.	do	do	Positive.
Control.	do	do	Negative.

TABLE No. VII.

January 25, the same sera as in Table No. VI again tested, with the following results:

IMMUNE SERUM NUMBER III.			
Dilution.	After 1 hour.	After 2 hours.	After 16 hours.
1:10	Positive	Positive. Marked precipitate.	Positive fluid cleared.
1:20	Negative	Negative	Considerable sediment. Fluid cleared.
NORMAL SERUM (FOR CONTROL).			
1:10	Negative	Negative	Considerable sediment. Fluid almost cleared.
1:20	do	do	Do.
Control.	do	do	Negative. Slight precipitate.
Control.	do	do	Do.

The same sera were tested with a 24-hour culture of the strain "Pest Avirulent" on this date. The results were as follows:

TABLE NUMBER VIII.

Dilution.		After 1 hour.	After 2 hours.	After 16 hours.
Immune Serum.	1:10	Positive	Positive	Positive.
	Control.	Negative		Marked precipitate.
Normal Serum.	1:10	do	Marked flocculent precipitate.	Do.
	Control.	Negative		Do.

Agglutination tests were made of the same horse's serum, the animal having received 10 agar cultures intravenously before this examination. The anti-infectious power of the serum at this time was such that 1 cubic centimeter protected 60 per cent of the inoculated white rats against fatal pest infection. The results are as follows:

TABLE No. IX.—*Immune serum Number III with strains "Maassen Alt" and "Pest Avirulent."*

"MAASSEN ALT."

Dilution.	After 4 hours.	After 20 hours.
1:10	Positive	Positive. Fluid cleared.
1:20	Negative	Moderate sediment. Fluid not clear.
1:40	do	Do.
1:80	do	Do.
Control.	Very slight precipitate of bacteria	Do.
Control.	Negative	Do.

"PEST AVIRULENT."

Dilution.	After 2 hours.	After 4 hours.
1:10	Positive	Positive. Fluid cleared.
1:20	Negative	Do.
1:100	do	Negative. Slight sediment.
1:1,000	do	Do.
1:2,000	do	Do.
Control.	do	Sediment. Fluid not clear.
Control.	do	Do.

The agglutination of *Pest immune serum Number IV*, obtained from a horse, was tested five months after its preparation. Its anti-infectious value was such that 1 cubic centimeter protected 90 per cent of the inoculated white rats against pest. The agglutination tests were made with the strain "Pest Virulent" (second transplant) at this time in dilutions of 1:10, 1:20, 1:100, 1:500, 1:1,000, with two controls. All were negative after four hours except in the dilution of 1:10, where a weak positive reaction occurred.

THE AGGLUTINATING PROPERTIES OF THE BLOOD IN IMMUNIZED HUMAN BEINGS.

A study of the agglutinating properties in the blood of a number of persons who had been vaccinated against plague, as well as of several cases who had suffered with the disease, is summarized in the following table. The blood was collected and tested from the vaccinated cases, from one week to ten days after the inoculation. It was taken from those convalescent from plague ten days after the subsidence of the fever. In the two cases in which it was obtained at autopsy, the patients had lived ten and fourteen days, respectively, after the onset of the disease. In order that the tests might be carefully and repeatedly made, from 10 to 20 cubic centimeters of blood was collected in the living cases from a vein of the arm. In the majority of instances reactions were at the same time performed with fresh, normal human serum. The sera were all tested within a day or two after the blood was drawn. A second transplant 24-hour culture of the virulent strain was employed in all the tests.

TABLE No. X.—*Tests of the agglutinating value of human immune sera.*

Case No.	Manner of inoculation.	Dilutions.	Results and remarks.
I	1/10 oese "Pest Avirulent;" 1 week later ⅛ oese "Pest Avirulent."	1:2; 1:5; 1:10; 1:20; 2 controls (no serum).	After 1 hour all negative. After 16 hours the precipitation of the bacteria is quite marked in all of the tubes but not so marked or of so dense a nature in the control tubes.
I	do	1:2; 1:5; 1:10; 1:20; 1:50; 1:100; 2 controls (no serum).	After 1 hour all negative. After 16 hours sediment in all the tubes but more marked in the tubes containing serum than in the control tubes. The same sera tested again the following day with the same results.
		1 control horse pest immune serum.	Positive after 1 hour.
II	⅛ oese "Pest Avirulent."	1:10; 1:20; 4 controls (no serum).	After 1 hour all negative. After 16 hours sediment in all the tubes; more marked in all tubes containing serum than in control tubes. The same sera were tested on the following day with similar results.
		2 controls with normal human serum, dilutions 1:10; 1:20.	
III	2 oesen "Pest Avirulent."	1:10; 1:20; 1:40; 1:80; 1:160; 2 controls without serum.	After 2 hours distinct precipitation of bacteria in dilution of 1:10; after 2½ hours precipitation appears also in dilutions of 1:80 and 1:160. Remaining tubes show no precipitation.
		1 control normal human serum, 1:20.	

TABLE NO. X.—*Tests of the agglutinating value of human immune sera*—Cont'd.

Case No.	Manner of inoculation.	Dilutions.	Results and remarks.
IV	2 oesen "Pest Avirulent."	1:10; 1:20; 1:40; 1:80; 1:160; 2 controls without serum.	After 2 hours faint precipitation of bacteria in dilution of 1:10. After 3 hours the same precipitation appears in the dilution of 1:20. The control tube with normal serum and the remaining tubes show no precipitation at this time.
		1 control normal human serum, 1:20.	
V	do	1:2; 1:10; 2 controls (no serum).	Moderate precipitation of bacteria after 2 hours in dilutions of 1:2 and 1:10. No sediment in control tubes.
		1 control normal human serum, 1:5.	Slight sediment of bacteria after 3 hours.
		1 control horse pest immune serum, 1:10.	Positive after 1 hour.
V	do	1:10; 1:20; 1:40; 1:80; 3 controls (no serum).	After 2 hours marked precipitate of bacteria in dilutions of 1:10 to 1:40. Controls negative.
		Control normal human serum 1:10.	Negative after 2 hours.
VI	do	1:2; 1:10; 1:20; 1:40; 1:80; 2 controls (no serum).	After 2 hours marked precipitate of bacteria in dilutions of 1:2 to 1:40.
		Control normal serum 1:10.	Negative after 2 hours.
VII	do	1:2; 1:10; 2 controls (no serum).	After 2¼ hours marked precipitate of bacteria in dilution of 1:2 and 1:10. Controls negative.
		Control normal human serum 1:5.	After 3 hours moderate precipitate of bacteria.
		Control horse pest immune serum 1:5.	Positive after 2 hours.
VII	do	1:10; 1:20; 1:40; 1:80; 1:160; 3 controls (no serum).	After 1 hour marked precipitate of bacteria in dilution of 1:40. After 2 hours marked precipitate in dilutions of 1:10, 1:20 and 1:80. Controls negative.
		Control normal human serum 1:10.	Negative after 2 hours.
		Control horse pest immune serum, 1:5.	Moderate precipitate after 2 hours.

TABLE No. X.—*Tests of the agglutinating value of human immune sera*—Cont'd.

Case No.	Manner of inoculation.	Dilutions.	Results and remarks.
VIII	One agar slant "Pest Avirulent."	1:5; 1:10; 1:20; 1:40; 1:80; 1:160; 1:320; 3 controls without serum.	After 2 hours no precipitate of bacteria in any of tubes. After 16 hours marked precipitate in dilutions of 1:5, 1:20, and 1:40.
		Control normal human serum, 1:5; 1:10; 1:20; 3 controls without serum.	After 2 hours moderate precipitation of bacteria in dilution of 1:10; after 4 hours in dilutions of 1:5 and 1:20.
VIII	do	1:2; 1:5; 1:10; 1:20; 5 controls without serum.	After 2 hours all negative. After 16 hours marked precipitation with clearing of fluid in dilutions of 1:5, 1:10 and 1:20 Not in 1:2.
		Control with human serum, 1:5; 1:10; 1:20. 3 controls (no serum).	After 2 hours all negative. After 16 hours marked precipitation with clearing of fluid in dilutions of 1:5, 1:10, and 1:20. Controls negative.
IX	do	1:2; 1:5; 2 controls (no serum).	All negative after 1 hour. After 16 hours dilution 1:5 marked precipitation. Other tubes negative.
		Control normal human serum I, 1:2; 1:10. Control normal human serum II, 1:2; 1:5.	Negative after 1 hour. After 1¼ hours flocculent precipitate strongly resembling agglutination appears in the dilution of 1:10 of one normal serum and in the dilution of 1:5 in the other. Precipitate disappears on shaking. Dilutions of 1:2 with each serum negative. After 15 hours dilutions of 1:10 and 1:5, heavy precipitate. Dilutions of 1:2 still negative.
X	do	1:2; 1:5; 2 controls (no serum).	All negative after 1 hour. After 16 hours moderate precipitate of bacteria in dilution of 1:5.
		Control normal human serum, 1:1: 1:5.	Negative after 1 hour.
X	do	1:2; 1:5; 2 controls without serum.	Negative after 1 hour. Moderate precipitate of bacteria in dilution of 1:5 after 16 hours.
		Control normal human serum, 1:2; 1:5.	Negative after 1 hour. Moderate precipitation of bacteria in dilution of 1:5 after 16 hours.
XI	do	do	Exactly the same results obtained as in Case X.
XII	do	1:2; 2 controls without serum.	Negative after 1¼ hours.
XIV	do	1:2; 1:5; 2 controls without serum.	Negative after 1 hour.

TABLE No. X.—*Tests of the agglutinating value of human immune sera*—Cont'd.

Case No.	Manner of inoculation.	Dilutions.	Results and remarks.
XV	One agar slant "Pest Avirulent."	1:2; 1:5; 2 controls (no serum).	After 1 hour and 10 minutes a flocculent precipitate began to appear in the dilution of 1:10. (Compare with Case IX reaction with normal serum.) Dilution 1:2 negative.
XVII	do	do	All negative after 1 hour. After 16 hours moderate sediment in dilution of 1:5.
		Control normal human serum, 1:2; 1:5.	Both negative after 1 and 16 hours.
XVIII	do	1:2; 1:5; 2 controls (no serum).	Exactly the same results as with Serum XVII.
XIX XX XXI XXIII	do	do	All negative after 1 hour. In Case XXI slight precipitate in dilution of 1:5 after 3 hours.
XXIV	do	do	All negative after 1 hour.
		Control normal human serum, 1:2; 1:5.	Negative after 1 hour. Slight precipitate in dilution of 1:5 after 3 hours.
XXV	do	1:2; 1:5; 2 controls (no serum).	All negative after 1 hour. Sediment in dilution of 1:5 after 16 hours.
XXVI XXIX XXXI XXXIII	do	do	All negative after 1 hour.
Plague case		do	Do.
Plague autopsies I and II.		do	Do.

A careful study of the above table brings us to the conclusion that in the majority of the instances no traces of agglutinins could definitely be demonstrated. In a few cases they perhaps were present in very small amounts.

In concluding the remarks on agglutination it may again be emphasized that excessive precautions must be taken to distinguish between pseudo and true agglutination in pest, and that apparently only in the organism highly immunized against plague infection do the agglutinins become developed in sufficient quantity to be of any practical importance either in the diagnosis of the infection or in the demonstration of the presence of an immunity. It also would appear from these experiments that the development of the anti-infectious substances in a plague immune serum is quite independent of the development of the agglutinins.

IX. THE BACTERICIDAL ACTION OF PLAGUE SERUM.

The German Plague Commission concluded that in pest immune sera specific bactericidal anti-bodies were present, the action of which was fully analogous to that of the protective substances which had been demonstrated by R. Pfeiffer to exist in cholera and typhoid infection. However, they made no experiments which demonstrated that such sera possessed a bactericidal action against the pest bacillus, although some experiments were performed which demonstrated their preventive action against infection and their curative value. Nevertheless, the opinion that plague immune serum exerts a bactericidal action against this organism apparently became generally accepted, although but little experimental work was carried on upon the subject.

In 1902, Kolle and Martini[67] performed experiments with guinea pigs and rats in which the animals were inoculated with from 1 to 2 cubic centimeters of pest immune serum and after twenty-four hours were inoculated intraperitoneally with from 2 to 3 oesen of pest cultures of moderate virulence, suspended in saline solution. Three or four hours after the inoculation of the bacteria, upon microscopical examination according to the method of Pfeiffer, of drops of the exudate from the abdominal cavity, the majority of the bacilli were found to be swollen, degenerated, and broken up. This phenomenon was not noted in control animals treated with normal serum, but pest bacilli of normal appearance were observed, which increased in number from hour to hour up to the time of the death of the animal. The abdominal exudate of the animals injected with immune serum was sometimes apparently sterile after twenty-four hours, although in these cases the few remaining bacteria usually multiplied and caused the death of the animal at a later time. Animals, such as rats, which had been actively immunized against pest by repeated subcutaneous injections of living, attenuated cultures, when injected intraperitoneally with pest strains of moderate virulence also exhibited the same bactericidal action toward the bacteria, but no anti-toxic action could be observed.

Markl[68] as a result of his observations found that the process of destruction of pest bacilli varied according to the virulence of the organism. When a culture of very great virulence was inoculated into the abdominal cavity of a guinea pig which had been treated with an immune serum, after thirty minutes a very extensive leucocytosis occurred and the bacteria were taken up by the phagocytes. Those bacteria which remained free, became agglutinated and grouped about the leucocytes. One hour after the injection of the immune serum he could find no extracellular bacilli in the peritoneal exudate and cultures made from it either remained sterile or developed only a few colonies. A leucocytosis also occurred in control animals without immune serum, but the bacilli remained in this instance extracellular and cultures of the abdominal exudate on agar produced a rich growth of bacteria. In further experiments on rats, where strains of different pathogenetic power were employed, the very virulent strains were taken up by the phagocytes through the action of the immune serum, while avirulent strains (those non-lethal in doses of 2 oesen) became dissolved in the abdominal cavity of the animal without the aid of the phagocytes. Strains of moderate virulence became partially destroyed by both of these means. The same mechanism was observed in passive immunization with serum, as was seen in animals actively immunized, either with killed or with living attenuated cultures.

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[67] *Deutsche med. Wchnsch.* (1902), **28,** 45.

[68] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1903), **42,** 244.

Kolle[69] in December, 1904, on pursuing further experiments of this nature, confirmed Markl's results. He also compared the method of action of plague, cholera and typhoid immune sera, testing the bactericidal action of plague serum *in vitro* after the method of Neisser and Wechsberg. He was unable to demonstrate any bactericidal reaction, in spite of the many variations in the experiments and the use of many different sera to supply the complement for the completion of the action of the amboceptors. Plague bacilli after treatment with the immune serum developed as plentifully in the culture media as they did in those instances in which they were treated with normal serum. Kolle and Hetsch employed fresh, normal serum from the pigeon, cow, horse, chicken, rabbit, donkey, and rat for supplying the complement. Upon investigating the question of whether the bacterial receptors of the plague bacillus were able to bind amboceptors in an immune serum, it was shown that such a binding did actually take place, and that the serum after being first treated with living plague bacilli lost in anti-infectious power, as was shown by experiments performed on rats. However, at the temperature of the ice box or when killed pest bacilli were substituted for living ones, binding of the amboceptors could not be demonstrated. Moreover, the union did not occur under the same quantitative relations as it does with typhoid and cholera immune serum. Kolle therefore concluded that the pest serum acts neither as a pure antitoxic serum, such as we see in diphtheria and tetanus, nor as a pure bactericidal one, such as we see in cholera and typhoid.

Skschivan[70] also obtained Pfeiffer's phenomenon in guinea pigs which were inoculated with 4 cubic centimeters of the Paris pest serum and sixteen to twenty hours later reinoculated with from 2 oesen to 1 agar culture intraperitoneally. When the serum was inoculated into the peritoneum, the bacteria became broken up in one-half hour. The control animals without serum died after one to two days while those inoculated with immune serum lived for from five to seven days. In the latter instance, in three cases, a periorchitis existed and the omentum was shrunken and hard.

In 1902, Wright and Windsor[71] showed that normal human serum was entirely without bactericidal action upon the pest bacillus and that sterilized cultures of this organism were not capable of abstracting a bactericidal element from such blood. In the following year Wright and Douglas[72] further showed that, while no bactericidal reaction was exerted against the plague bacillus by normal human serum, such serum evidenced a distinct opsonic action against this organism. On the other hand Row[73] maintained that the serum of plague convalescents possessed remarkable bactericidal properties. He found that while in hanging drop preparations, the *Bacillus pestis* flourished in the serum of normal individuals, when a quantity of serum from a plague convalescent was infected with this organism the latter was destroyed after twenty-four hours. The growth of the plague bacillus was said to be inhibited, in the case of blood taken from patients in the early stage of the disease or from those tending to recover. In a later paper [74] he pointed out that this bactericidal reaction of the pest bacillus resulted from the action of immune bodies plus complement, and that the action of plague serum could be suspended by the usual method of inactivation and it could again be reactivated by the addition of fresh complement. He claimed that Roux's serum which had been inactivated, could be reactivated by fresh complement obtained from either man, the dog, rat, or monkey. However, Row's experiments seem inconclusive and unconvincing owing to the technique employed.

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[69] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1904), **48,** 371.

[70] *Centrbl. f. Bakteriol. Orig.* (1903), **33,** 271.

[71] *J. of Hyg.* (1902), **2,** 385.

[72] *Proc. Roy. Soc. Lond.* (1904), **73,** 130.

[73] *Brit. Med. Journ.* (1902), 1895.

In 1906, Lamb and Foster [75] again emphasized the fact that not only normal human serum, but also that of other mammals, was devoid of any bactericidal action on *Bacillus pestis*.

Schouroupoff,[76] who demonstrated that the pest bacilli which were injected into animals immunized against pest, disappeared after a very short time, concluded that the antipest serum is also partly bactericidal in its action.

Löhlein [77] very recently communicated the results of his studies upon phagocytosis in pest and anthrax to the Association of Microbiology at Berlin. He found that while in the animal body almost no phagocytosis of virulent pest strains occurred, *in vitro* the organisms were taken up by the carefully washed white blood corpuscles of the most susceptible experimental animals and he further demonstrated that while phagocytosis of the virulent pest organism was not dependent upon the action of dissolved opsonic substances, such action was promoted by normal guinea pig serum. The same phenomena were observed with dilutions of specific plague serum. In the case of undiluted serum, or in its lower dilutions, the serum was strongly agglutinative, so that the result was obscured. Lohlein, in accord with the experiments of Markl, found that *in vitro* the most marked prevention to the growth of virulent pest bacilli occurred when immune serum and leucocytes were placed in contact with the pest bacilli. Upon injecting virulent pest germs into the "prepared" abdominal cavity of guinea pigs, a marked phagocytosis of the bacteria also occurred. A rapid diminution of the bacteria at first, as well as of the animal cells in the abdominal cavity, also occurred upon injecting these organisms into the abdominal cavity of a *normal* guinea pig or rat. A dissolution of the bacteria was not observed, but the organisms were taken up by phagocytic cells in different portions of the abdomen and in the omentum. Later, a marked increase in the number of leucocytes in the abdominal cavity took place and especially, the bacteria became increased in number. These organisms, which were termed "bacteria of the second generation," increased up to the time of the death of the animal. The bacteria which appeared after the "negative" period were not usually taken up by phagocytes. This failure of the leucocytes to ingest them was evidently not due to an injury to the phagocytic properties of these cells, as was demonstrated by the further injection of staphylococci into the abdominal cavity, the latter organisms undergoing active phagocytosis. Lohlein thought this phenomenon might be explained either upon the assumption of the presence of a soluble substances such as an aggressin, in the sense in which Bail uses the term, or by assuming that the bacteria which appeared later in the abdominal cavity, surrounded by capsules, possessed a negative chemotactic action. Possibly both of these factors played a rôle, although he was unable to determine the question definitely. He also found, as Denys and Tartakowsky had done, that where immune serum was injected into the abdominal cavity of a guinea pig, shortly after the introduction of the organisms, phagocytosis in addition to agglutination of these bacteria of the second generation occurred.

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[74] *Brit. Med. Journ.* (1903), 1078.

[75] *Lancet* (1906), **171,** 9.

[76] *Arch. des Sci. Biologiques* (1905), **11,** 196.

[77] *Centrbl. f. Bakteriol. Abt. I* (1906), **38,** 32.

After a study of the experiments which had been performed in relation to the bactericidal action of the pest immune serum *in vitro,* it appeared to me, to judge from the careful experiments of Kolle and Hetsch on pest immune serum, that this question was definitely settled as far as the methods employed were concerned. The experiments of Wright and Lamb and my own already referred to, relating to the bactericidal reaction *in vitro* of normal human and other mammal's blood, also appeared conclusive. However, in order further to complete the evidence, the following additional experiments, consisting of the examination of the bactericidal power of horse's perfectly fresh pest immune serum, as compared with horse's fresh normal serum, were undertaken. I also studied the bactericidal power of horse's inactivated pest immune serum, after the addition of perfectly fresh monkey serum to supply the complement. The horse's immune serum in the latter instance was inactivated by heating for one hour at 56° C. The experiments were performed in the following way:

In test tube I were placed 1 cubic centimeter of perfectly fresh, horse's immune serum (without the addition of carbolic acid) + 1 cubic centimeter of a suspension of an agar culture of the virulent pest bacillus in bouillon, in a dilution of 1 to 50,000.

In test tube II were placed 1 cubic centimeter of perfectly fresh, normal horse serum + 1 cubic centimeter of a suspension of an agar culture of the virulent pest bacillus in bouillon in a dilution of 1 to 50,000.

After thorough mixing of the contents of each tube they were both placed for three hours in the incubator at 37° C., when sets of agar plates were prepared. No difference in the bactericidal action of the two sera could be distinguished.

In the second series of experiments, in test tube I were placed 1 cubic centimeter of the inactivated, horse's immune serum + 0.5 cubic centimeter of the suspension of the bacilli + 0.5 cubic centimeter of monkey's fresh normal serum in a dilution of 1: 10.

In test tube II were placed 1 cubic centimeter of horse's inactivated, normal serum + 0.5 cubic centimeter of a suspension of the bacilli + 0.5 cubic centimeter of monkey's fresh, normal, serum in a dilution of 1: 10.

In test tube III were placed 0.5 cubic centimeter of the supension of the bacilli + 1.5 cubic centimeters of saline solution.

In test tube IV, 0.5 cubic centimeter of a suspension of the bacilli + 0.5 cubic centimeter monkey's normal serum + 0.5 cubic centimeter saline solution.

These experiments were performed in dilutions of the serum of 1:2, 1:10, 1:100, and 1:500. No marked difference could be discovered in the number of colonies in the plates prepared from the suspensions of immune serum and from that of the normal serum.

The success of the reaction in experiments of this nature, providing of course that the serum to be tested possesses a bactericidal power, obviously depends upon the selection of an animal serum containing complement with receptors suitable to unite with the amboceptors in the immune serum. As has been mentioned, Kolle and Hetsch in their experiments employed the sera of the pigeon, cow, horse, chicken, rabbit, donkey, and rat to furnish the complement.

However, it was demonstrated by the following additional experiments that my failure, in the experiments which I have just described, to obtain a bactericidal reaction or to demonstrate any other injurious effect on the plague bacillus resulting from the action of the pest immune serum on this organism was not due to the fact that I had not found a suitable complement for the amboceptors.

A horse's pest immune serum (not inactivated), *which at the time in doses of 1 cubic centimeter was able to protect against fatal pest infection about 90 per cent of the rats inoculated with it,* was mixed with perfectly fresh rat serum and its bactericidal value tested according to the method, *in vitro,* which has already been described. In order that the phenomenon of the deflection of the complement by amboceptors, as described by Neisser and Wechsberg, might not interfere with the reaction, the experiments were also performed with varying amounts of the horse's immune serum and fresh rat serum. However, no difference could be detected between the results obtained with these experiments and with those performed in the same manner with normal horse serum plus fresh rat serum.

These experiments and those of Kolle and Hetsch already outlined appear to demonstrate that the pest immune serum which is known to possess anti-infectious power in the animal, possesses *in vitro* no bactericidal action whatever, that is similar to that possessed, for example, by typhoid immune serum. It is also clear that the pest bacilli are not only not killed by the immune serum, but that they remain alive and are capable of subsequent development. Therefore, it is evident that some other factor must play an important rôle in the ultimate destruction of the inoculated bacilli in the body of an animal either passively immunized by the injection of such a serum or actively immunized by vaccination, and according to the conceptions of Metchnikoff it might be argued that since the serum in the test tube is apparently without injurious action upon the pest bacilli, the phagocyte must be the additional factor which is necessary to render harmless and to destroy the organism in question.

However, before accepting this hypothesis, it seemed advisable further to investigate not only what action the serum has upon the life of the plague organism, but also what action the organism has upon the immune serum. From the extensive work performed by Ehrlich, Morgenroth, Pfeiffer, Wassermann, Kolle, and their co-workers, we know that a union occurs *in vitro* between the specific substances of a serum, such as antitoxine or bacteriolysine, and the homologous bacterial antigen. Although the union of these two substances follows a different law, it is possible to show that such a binding actually does take place and that the antitoxic serum loses in value after combination with toxine and the bactericidal one diminishes in its specific effect after treatment with its corresponding bacterium.

With a view of further elucidating the subject, this problem was undertaken with the plague bacillus and pest immune serum; the experiments being performed in a somewhat similar manner to those previously carried on in this laboratory in 1904 with the cholera organism. The pest immune serum was first carefuly tested for its anti-infectious power on rats and the amount determined which would protect about 90 per cent of the animals inoculated with it, against the subsequent injection of a lethal dose of plague bacilli. (See Series 53, p. 286.) Fifteen cubic centimeters of this pest serum were then mixed with the living bacteria obtained from fifteen 48-hour agar slant cultures of the virulent pest strain. The mixture was placed in the incubator for two hours at 37° C.; carbolic acid to 0.5 per cent was then added and the mixture heated for two hours at 46° C. and then thoroughly centrifuged. The clear fluid above was then drawn off from the sediment of bacteria. After the sterility of the serum had been demonstrated, its anti-infectious value was now for a second time tested on rats and it then was found that the serum no longer protected these animals in the same amounts as it did previous to its treatment with the baceria, 70 per cent of the rats inoculated with the same dose succumbing when subsequently infected with pest.[78]

It follows from this that a binding of at least a portion of the amboceptors of the pest immune serum to the receptors of the plague bacillus had occurred, and although the bacteria in question were not killed by the serum, nevertheless, a reaction *in vitro* between the serum and the organism had occurred. (For the details of these experiments see Series 53, p. 286.) These results are confirmatory of those already referred to and obtained by Kolle and Hetsch.

For the further study of the action of plague immune serum I performed other experiments in the abdominal cavities of guinea pigs, the details of which may be seen in Series 63, 64, and 65 (pp. 268, 270, and 271). Upon injecting the strain "Pest Virulent" into the peritoneal cavity of a guinea pig immunized against plague either by previous vaccination or by the injection of a dose of pest immune serum, it was found that Pfeiffer's phenomenon as observed in the case of the cholera organism in the cholera immune animal did not occur. The virulent organism in question did not undergo dissolution and only with the very avirulent strains ("Maassen Alt" and "Pest Avirulent") did the organisms finally become swollen or disintegrated. It is true that shortly after the inoculation of the virulent strain in the immunized animal, a disappearance of the bacteria from the abdominal cavity usually occurred, and that also at first but few animal cells were encountered in the abdominal exudate. Upon investigating the fate of the bacteria by killing the animals at different periods of time after the inoculation, it was found that shortly after the injection, both in the case of animals immunized against pest and in that of normal animals, the majority of the bacteria had been carried to or made their way to the walls of the cavity and particularly to the omentum, to the surface of which they had become adherent. Here many of them were taken up by the phagocytic cells. After a short period, the leucocytes became more abundant in the abdominal exudate and many of them were seen to contain bacteria. In many cases, in the immunized animal, the leucocytes seem to possess positive chemotaxis for the bacteria, judging from the manner in which the latter are grouped about them.

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[78] In performing experiments of this nature precautions must be taken to ascertain if sufficient amounts of dissolved receptors of the bacilli (artificial aggressins) remain in the serum to influence the course of the infection in the animal.

In the case of nonimmune animals the pest bacilli outside of the cells increase in number up to the time of the death of the animal. The majority of the bacteria that are found to exist free in the cavity after the negative phase, are short, bipolar staining bacilli which seem to possess capsules; a smaller number of large bacilli, frequently showing involution forms, are also encountered. After the negative phase in the case of the immunized animal, the leucocytes usually become much more numerous in the abdominal cavity. The phagocytosis of the bacteria continues, both by the cells in the omentum, and by those free in the abdominal cavity, until very few free bacilli remain. However, as has been mentioned, in the nonimmune animals the bipolar staining organisms, which do not appear to be taken up by the leucocytes, increase up to the time of the death of the guinea pig. (See Series 63, 64, and 65 (pp. 268, 270, and 271.)

Löhlein,[79] whose article was published while my experiments were in progress, also encountered these bipolar staining organisms. These he very appropriately terms "bacteria of the second generation," and he calls attention to the fact that they are seldom seen to undergo phagocytosis. This phenomenon as mentioned he explains either upon the assumption that some such substance as the aggressin of Bail may be present, or that perhaps the capsules of the bacteria act in a negatively chemotactic manner. My own idea is that these organisms are negatively chemotactic for the leucocytes, but not because they possess aggressins in the sense which Bail uses the term, but because they have not been changed chemically by the action of the immune serum—that is, these bacteria have either entirely escaped the action of the plague amboceptors, or a sufficient portion of their receptors have not been bound to enable the leucocytes to act positively chemotactic toward them and to engulf them; the phagocyte usually ingesting only the organism which has previously been affected by the immune serum; it is also possible that the encapsulated organisms are not readily hydrolyzed.

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[79] *Loc. cit.*

Therefore, it is obvious that *in vitro* the amboceptors of plague immune serum unite with the receptors of the organism and that in the body of the animal the process of destruction is carried on further by the phagocytes, which engulf the bacteria which have been so acted upon. It is also evident that the bacteria are not killed in the test tube by the immune serum alone. Does the leucocyte accomplish this action, or is the organism killed in the abdominal cavity by other influences before undergoing phagocytosis? That the latter hypothesis is not always true I have been able to demonstrate by transplanting to the surface of agar media loops of the abdominal exudate which contain phagocytes inclosing bacteria. The organisms under these circumstances have sometimes been observed to have increased within the cells and in some instances to have burst the leucocyte and partially to have escaped from it.

It therefore seems that after the bacillus has been prepared for the action of the leucocyte by the immune serum, the leucocyte does play a part in the digestion and ultimate destruction of the organism. On the other hand, that the phagocytes in nonimmunized animals do not to any extent take up plague bacilli seems equally clear and this opinion may be confirmed by the study in plague infections of smears or sections from plague buboes or abscesses in other portions of the body. Here, while many bacteria are encountered in the endothelial cells, but very limited or no evidence of phagocytosis by polymorphonuclear leucocytes is encountered.

The destruction of the plague bacillus is then effected by the immune animal in a manner partly in accord with the humoral theory of Buchner and partly in accord with the phagocytic one of Metchnikoff. The action of the serum in its protective effect upon the animal is neither antitoxic nor bactericidal, but has been termed anti-infectious; that is, it is a serum possessed with the power of preventing infection.

X. OPSONIC ACTION OF PLAGUE SERUM.

As is now generally known, Wright and Douglas[80] in 1903 proposed the term "opsonins" to designate the elements in the blood which modified the bacteria in a manner which rendered them a ready prey to the phagocytes. Their important article, at the time of its appearance, did not excite the attention which it merited, but in 1904 and 1905 they, together with Bullock,[81] undertook further studies relating to the variations of the opsonic content of the blood in certain infections and explained the practical results to be obtained from the observation of the opsonic index. These publications attracted wide attention. During the past year many articles have appeared relating to the presence of opsonins in different infections and of the practical results to be obtained by increasing the opsonic index through the injection of bacterial vaccines. While some of this work has been done by trained investigators, much of it has been performed by men who may be considered new to the field and it is questionable whether all of the claims of the *practical* results obtained with the various infections will be justified by more careful work in the future. There are many sources of error in determining the opsonic index, and the personal equation is of considerable importance when the method described by Wright is employed.

Among the more important articles on this subject which have appeared during the past year, besides those of Wright, may be particularly mentioned those of Hektoen[82] and his pupils and of Simon, Rosenow and Porter[83] in the United States, and of Muir and Martin in Great Britain.

A discussion of the entire subject can not here be entered into. Hektoen and Bullock believed that the opsonins might be regarded as distinct from the other anti-bodies. However, it does not seem to me that this has been at all *conclusively* established, and in my experimental work on the subject I have not been able to convince myself that in the "opsonic" action in plague we have to do with any other factors than the binding of the receptors of the bacteria by amboceptors of the serum and of the union of the complement in the usual way, as in bacteriolysis, and believe that it is in this manner that the bacteria are prepared for phagocytosis. However, obviously the complement does not always necessarily cause lysis, although it always enters into the reaction.

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[80] *Proc. Roy. Soc. Lond.* (1903), **72,** 357.

[81] *Ibid.* (1904), **73,** 128, and *Lancet* (1905), **2,** 1598 and 1603. These articles contain the other important references to Wright's work.

[82] *J. Am. Med. Ass.* (1906), **46,** 1407, and *J. Infect. Dis.* (1906), **3,** 434.

[83] *J. Am. Med. Ass.* (1906), **47,** 1722.

It seems not unlikely that in some of the experiments performed in the study of the opsonins it is sometimes the action of the complement alone and sometimes, though more rarely, that of the immune body which has been considered to represent the opsonic action. The recent work of Simon[84] and his associates which argues against the specific character of opsonins, supports these views, as does also the work of Muir and Martin.[85] These latter authors have tested the three chief varieties of immune bodies (amboceptors), namely, (1) those obtained by the injection of red blood corpuscles, (2) serum, and (3) bacteria, and have found that in each case the combination of receptors plus immune bodies removes the opsonins of normal serum. Moreover, it seems to me that another very important factor in favor of the hypothesis that opsonic action consists of the binding in combination of amboceptors and complement is furnished by a study of the action of the plague bacillus and plague serum in relation to the so-called phenomenon of Bordet and Gengou[86] of the fixation of the hæmolytic complement.

I also have been able to show that plague immune serum constantly produces the phenomenon and we can therefore reason from this fact and from the work of Muir and Martin that some complement as well as amboceptor enters into the opsonic reaction in plague, since we know that the complement is only bound according to the phenomenon of fixation when a union of specific amboceptor and receptor has occurred and that, conversely, the phenomenon only takes place when the complement is actually bound. (See pp. 327 and 328 for the details of these experiments.)

However, leaving aside the discussion of whether the opsonins really represent new anti-bodies of a different character from those we are familiar with, we may certainly say that plague immune serum possesses opsonic power since it prepares the organisms for phagocytosis, and we may add that with the plague bacillus the result of the chemical reaction is somewhat different from that which is observed in true bacteriolysis of certain other microörganisms.

I have also investigated the question of whether the sera of human beings and animals vaccinated against plague develop a higher opsonic index against the plague organism than is possessed by normal human and animal sera. Some of the guinea pigs and monkeys in which the reaction was tested had been shown to resist plague infection after the vaccination. The blood was usually tested ten days after the vaccination or test of immunization. The method of experimentation has been as follows:

A non-immunized guinea pig is inoculated intraperitoneally with 10 cubic centimeters of a sterile suspension of aleuronat. After from twelve to sixteen hours the animal is killed with chloroform. The abdominal exudate, if sterile, is collected and mixed with an excess of 0.1 per cent of ammonium oxalate solution in 0.85 per cent sodium chloride solution (as proposed by Simon,)[87] and thoroughly centrifuged. The packed corpuscles are then washed several times in excess of saline solution and each time recentrifuged. In performing the tests one volume of leucocytes plus one volume of a saline suspension of the pest bacteria plus one volume of the serum to be tested are thoroughly mixed and incubated for thirty minutes at 37° C. After this time smears are prepared and stained with methylene blue. Two hundred leucocytes were counted and the average number of bacteria ingested determined. Control experiments with normal sera of the same species of animal were prepared as well as a control showing the nonphagocytic power of the washed leucocytes. The method of counting the phagocytic cells as suggested by Simon rather than the number of bacteria in each phagocyte as recommended by Wright, appears somewhat simpler and the error of variation between repeated counts with it was in some instances not so great. However, which of these methods will eventually prove to be the more reliable in determining the actual opsonic index I am not prepared to state.

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[84] *J. Exp. Med.* (1906), **8**, 651.
[85] *Brit. Med. Journ.* (1906), 1783.
[86] *Ann. d. l'inst. Pasteur* (1901), **15**, 289.

A few experiments were also performed in determining the opsonic action of vaccinated animals by another method which, however, was not found to be as satisfactory as the one already described.

In these experiments the animals to be tested were first inoculated intraperitoneally with a suspension of the strain "Pest Virulent" and after thirty-five minutes to one and one-half hours, drops of the abdominal fluid were drawn off and smears prepared and stained. The average number of bacteria contained in 50 leucocytes was then counted.

While the animals which had been vaccinated by this method usually showed an increased opsonic index over that of normal animals, frequently the number of leucocytes in the abdominal cavity as well as the number of bacteria was very small. The explanation of this phenomenon has already been discussed above. The difficulties in the technique were therefore increased and further experiments were not performed with this method because of the superiority of the first one described. In the experiments in Series 61 (p. 267) the opsonic index of the animals before vaccination was unfortunately not determined and hence the results are not as valuable for comparison.

Carrying out experiments in this manner, the details of which are described in Series 61 and 62 (pp. 267 and 268), it has been possible to demonstrate that the blood of an individual or animal vaccinated and immunized against pest usually shows an increased opsonic index. However, that this was not invariably the case may be seen from the tables given in Series 61 and 62. The opsonic power apparently varied somewhat according to the virulence of the strain employed, the index usually being higher with the avirulent strain. No attempt was made to demonstrate the negative phase of the reaction or to plot their curves, and as the normal opsonic action is known to vary considerably from time to time, this may account for the apparent absence of the increase of the index in some of the cases at the time of the examination of the serum. Moreover, the reaction may not in some instances have reached its maximum or it may in others have begun to decline. However, the general law may be determined from the study of the entire series of experiments.

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[87] *Johns Hopkins Hosp. Bull.* (1906), **17**, **27**.

In conclusion it may then be stated that plague immune serum acts neither as an antitoxic serum nor as do other bactericidal sera, but its action may be said to be anti-infectious and opsonic in nature.

EXPERIMENTS.

Series 61.—*Determination of the opsonic index in guinea pigs.*

The animals were inoculated with 1 cubic centimeter of a suspension of the strain "Pest Virulent" in saline solution. Thirty-five minutes to one and one-half hours after the injection, drops of the abdominal fluid were drawn off and smears prepared and stained. The average number of bacteria contained in fifty leucocytes was then counted. Unfortunately, the opsonic index was not determined before the animals were vaccinated and therefore the experiments were performed in normal as well as in vaccinated animals.

Animal No.	How immunized.	Opsonic index of strain "Pest Virulent" after 1½ hours.
1977 (vaccinated)	Vaccinated with 2 cultures "Pest Avirulent." Immunity tested later.	11.3
2273 (normal)		9
1980 (vaccinated)	Vaccinated with 2 cultures "Pest Avirulent." Immunity tested later.	8
2282 (normal)		4.6
2154 (vaccinated)	1 agar slant "Pest Avirulent." Immunity tested later	8
2162 (vaccinated)	do	5
2861 (normal)		8

Series 62.—*Determination of the opsonic index in human beings, guinea pigs and monkeys.*

This series comprised the examination of the opsonic index in both human beings and animals vaccinated against pest. The opsonic index was tested against the strain "Pest Virulent" both before and after the immunization. In a few instances, after immunization, the opsonic index was also tested with two other strains of the pest bacillus, as may be seen from the table. One-tenth cubic centimeter of the serum, 0.1 cubic centimeter of a thoroughly washed suspension of guinea pig's corpuscles, and 0.1 cubic centimeter of a suspension of the organism "Pest Virulent" were thoroughly mixed and the resulting suspension placed at 37° C. for thirty minutes. Smears were then prepared and stained, two hundred leucocytes being counted.

SERIES 62—Continued.

Number.	Opscnic index for "Pest Virulent" before inoculation.	Vaccinated with—	Opsonic index for strain "Pest Virulent" tested 10 days after inoculation.	Opsonic index for strain "Avirulent Manila" tested 10 days after inoculation.	Opsenic index for strain "Pest Avirulent."
Human case 55	0.4	One 24-hour agar culture "Pest Avirulent"	2	--------	--------
Human case 56	.48	do	.7	--------	--------
Human case 58	.89	do	.8	--------	--------
Human case 59	.64	do	1.7	--------	--------
Human case 60	.4	do	.7	--------	--------
Human case 61	.52	do	1.4	--------	--------
Human case 62	1.2	do	.9	--------	--------
Human case 64	4.6	do	5.8	--------	--------
Monkey 2666 (vaccinated).	.34	1 culture killed "Pest Virulent." Immunity tested later.	.7	--------	--------
Monkey 2717 (vaccinated).	.64	One 24-hour culture "Pest Avirulent." Immunity later tested.	2	--------	--------
Monkey 3061 (normal).	1.	--------	.8	--------	--------
Guinea pig 2634 (vaccinated).	.42	One 24-hour culture "Pest Avirulent." Immunity tested later.	1.8	--------	--------
Guinea pig 2680 (vaccinated).	.6	1 culture of killed "Pest Virulent." Immunity tested later.	1.7	--------	--------
Guinea pig 3062 (normal).	.5	--------	.7	--------	--------
Guinea pig 2636 (vaccinated).	.48	One 24-hour culture "Pest Avirulent." Immunity tested later.	.4	1	5.4
Guinea pig 2648 (vaccinated).	.6	One 24-hour culture of "Maassen Alt." Immunity tested later.	2.4	3.2	1.8
Guinea pig 2639 (vaccinated).	.43	One 24-hour culture "Pest Avirulent." Immunity not tested.	.9	1.2	1
Guinea pig 3053 (normal).	.51	--------	.4	.46	.62
Guinea pig 2231 (normal).	1.8	--------	2	--------	--------
Guinea pig 1975 (vaccinated).	1.4	Two 24-hour cultures "Pest Avirulent." Immunity tested later.	4.3	--------	--------
Guinea pig 1976 (vaccinated).	2	do	3.1	--------	--------

SERIES 63.—*Experiments relating to the mechanism of the action of plague immune serum.*

Guinea pigs numbered 2152 and 2153 which had been immunized by plague vaccination and numbers 2757 and 2758, which were not immunized, all received intraperitoneally 1 full oese of the strain "Pest Virulent" suspended in 1 cubic centimeter of saline solution. Guinea pigs numbered 2152, 2153, and 2757 each also received intraperitoneally, at the same time, 1 cubic centimeter of pest immune serum, and guinea pig number 2758, 1 cubic centimeter of saline solution. Drops of exudate were taken from the abdominal cavity at intervals of from twenty minutes to one and one-half hours after inoculation. Some of these

SERIES 63—Continued.

were dried and stained and others were examined in a fresh condition. On microscopic examination there was no evidence whatever of bacteriolysis either in the fluid from the animals immunized and inoculated with immune serum or in that from the control animal (number 2758) although there was a diminution in the number of the bacteria which had been inoculated in the exudates from all. Those organisms which were present stained naturally and presented no evidence of degeneration.

The leucocytes were much more numerous in the preparations from animals numbered 2152 and 2153, fewer in number in those from animal number 2757 and still more scanty in those from animal number 2758. At a later period a still more marked reduction of the number of the bacteria in the abdominal exudate from all the animals occurred. This reduction, however, was more marked in the animals which had received the serum. On the following morning the guinea pigs were all killed with chloroform and the abdominal cavity in each instance immediately opened and its contents examined. In guinea pig number 2152 there was almost no local reaction apparent about the point of inoculation. The abdominal cavity contained a small amount of a tenacious fluid. Cover slips from this exudate stained with methylene blue showed a considerable number of polymorphonuclear and large mononuclear leucocytes, the latter variety predominating. None of the leucocytes contained any bacteria and no bacteria were found free in the exudate. A small portion from the edge of the omentum was cut off and rubbed over a glass slide and the preparation stained with methylene blue. This specimen showed innumerable polymorphonuclear leucocytes and large, mononuclear, endothelial phagocytes, the majority crowded with pest bacilli. There were a number of free organisms, but these were swollen and frequently stained irregularly and usually faintly so in color. They showed no capsules and no bipolar staining. Many appeared as involution forms. It seems likely that a number of these organisms have been extruded from cells or that the cells have burst and become destroyed and the bacteria have escaped. This opinion is supported by the fact that many bacilli with a similar appearance were seen within degenerating cells or lying about them.

It appears evident that the bacteria have become acted upon in some manner by the immune serum so that the cells of the omentum to which the bacteria have been attracted are enabled to pick them up in large numbers. It would seem that the great reduction in the number of bacteria in the abdominal cavity during the first hour after inoculation is not due to the fact that the bacteria have been destroyed but because they have been attracted to the omentum as to a sponge. That the bacteria inside the leucocytes were not all dead was demonstrated by placing some of the leucocytes containing ingested bacilli upon agar cultures. It was then found that when the leucocyte was removed from its natural conditions in the animal body the bacteria sometimes apparently multiplied within the cell and finally burst and destroyed it. The phagocytic cells of the omentum evidently play an important part in pest immunity. In guinea pigs numbered 2153 and 2757 very much the same conditions were observed as in number 2152. The cells of the omentum perhaps contained not so many bacteria and there were a few leucocytes in the abdominal cavity which contained a few ingested bacilli. In number 2757 there was not as much exudate present as in number 2152. The local reaction about the point of inoculation was also more marked in guinea pig number 2757, there being a hæmorrhagic area about 2 cubic centimeters in diameter about the point of inoculation. In guinea pig number 2758 there was a very marked local reaction about the point of inoculation with extensive hæmorrhages in the tissues. In this animal there was practically no fluid in the abdominal cavity although there were some flakes and masses of fibrin containing a fair number of

SERIES 63—Continued.

leucocytes and there were only a few bacteria and a moderate number of leucocytes which contained bacteria in the abdominal cavity. These free organisms, however, took a distinct bipolar stain and some of them appeared capsulated. Films made by rubbing portions of the omentum upon glass slides and staining the preparations showed numerous polymorphonuclear leucocytes and large mononuclear phagocytes. A number of these contained bacteria, but not nearly so many cells had ingested bacteria as in the case of guinea pigs numbered 2152, 2153, and 2157, neither was the number of bacteria in the cells so great. In the abdominal exudate of these animals and in the omentum there was a distinct reduction in the number of the bacteria inoculated and it would appear that the organisms had invaded further into the tissues and to other organs such as the spleen and even the blood. The slight evidence of phagocytosis observed in this animal would appear to be due to the fact that the bacteria had not been acted upon by immune serum.

SERIES 64.—*Relating to the mechanism of the action of plague immune serum.*

Guinea pig number 2852: One agar slant culture of "Pest Virulent" from guinea pig number 2764, second transplant, was suspended in 1 cubic centimeter of saline solution. This suspension together with 1 cubic centimeter of pest immune serum was injected into the abdominal cavity of the animal. Microscopic examination of drops of fluid from the abdominal cavity after twenty minutes showed almost complete disappearence of the bacteria. There were numerous large mononuclear and polymorphonuclear cells present, none containing bacteria. After fifty minutes there was still no evidence of bacteria in the exudate, although the leucocytes were more numerous. The animal was killed one and one-half hours after inoculation. The omentum and posterior abdominal walls showed enormous numbers of bacteria, a large number of which had been ingested by leucocytes.

Guinea pig number 2853: One agar slant culture of "Pest Virulent" from guinea pig number 2852, second transplant, was suspended in 2 cubic centimeters saline solution. The animal was inoculated intraperitoneally with this suspension. Microscopic examination of drops of fluid examined from the abdominal cavity after twenty minutes showed numerous bacteria, many in groups. Comparatively few leucocytes were present. After fifty minutes apparently not much change had taken place. There were still many bacteria in the exudate and a few cells containing organisms. The animal was killed after one and one-half hours. A few leucocytes were found in the abdominal exudate; a number of these contained bacteria. In smears from the omentum enormous numbers of bacteria were found. There was little evidence of phagocytosis.

Guinea pig number 2593, *immune guinea pig:* Inoculated intraperitoneally with 1 agar slant culture of "Pest Virulent" from guinea pig number 2764, second generation, suspended in 2 cubic centimeters saline solution. Drops of fluid taken from the abdominal cavity after twenty minutes showed a few cells and a few free bacteria. After fifty minutes, numbers of large mononuclear cells were visible in the exudate, many of these were packed with bacteria, others contained bacteria lying about them. These organisms did not stain as deeply as normal bacteria. The animal was killed one and one-half hours after inoculation. An extensive phagocytosis of the bacteria by the cells of the omentum was found to have occurred. But there were also large numbers of bacteria which were not ingested.

Guinea pig number 2854: One-half agar slant culture "Pest Virulent" (from guinea pig number 2764, second transplant) was suspended in 0.5 cubic centimeter saline solution. To this suspension was added 0.5 cubic centimeter of pest

SERIES 64—Continued.

immune serum and the mixture injected into the abdominal cavity. Microscopic examination of drops of fluid after twenty minutes showed numerous bacteria with but few leucocytes. No evidence of phagocytosis was present. After sixteen hours the animal was killed. A small amount of sticky fluid was encountered in the abdominal cavity, containing numerous polymorphonuclear leucocytes, the great majority of which did not enclose bacteria. In the few which had ingested pest bacilli, the organisms appeared degenerated. Very few free bipolar staining organisms were present. In the omentum evidences of an extensive phagocytosis existed. However, there were still a number of free bacteria. Some of the bacteria had apparently invaded other organs of the body.

Guinea pig number 2855: One-half agar slant culture "Pest Virulent" (from guinea pig number 2764, second transplant) suspended in 1 cubic centimeter of saline solution was inoculated into the abdominal cavity. Microscopic examination of drops of fluid from the abdomen after twenty minutes showed numerous bacteria and comparatively few cells. After one hour a few large mononuclear cells containing bacteria were present. The number of bacteria was considerably diminished. The animal was killed after sixteen hours. Very few bacteria were found in the abdominal exudate. Upon the omentum and surface of the abdominal walls innumerable bacteria were observed. There were moderate numbers of leucocytes present, some of which contained organisms. There were also a number of free bacteria which took the bipolar stain and appeared encapsulated.

SERIES 65.—*Relating to the mechanism of the action of plague immune sera.*

Guinea pigs numbered 2751, 2860, 2862, 2864, 2867, 3050, and 3059 were all inoculated intraperitoneally with 1 cubic centimeter of pest immune serum, mixed with 1 cubic centimeter of a suspension of pest bacilli of the several strains "Pest Virulent," "Maassen Alt," and "Pest Avirulent." Guinea pigs numbered 2863, 2868, 3051, and 3060 were employed for controls and were inoculated with a suspension of the bacteria to which was added 1 cubic centimeter of saline solution in place of immune serum. Drops of fluid from the abdominal cavities of the animals were examined from time to time, from five minutes to one hour after the inoculation, the animals also being killed at various periods after the inoculation. These examinations demonstrated that the "Avirulent" strains of pest bacilli do to a certain extent become swollen and undergo degeneration under the influence of plague immune serum, outside of the phagocytes.

However, only a portion of the bacteria inoculated showed these changes, and a typical Pfeiffer's phenomenon with granular destruction of the bacilli does not occur, the degeneration manifesting itself by swelling of the bacilli and their poor and irregular staining reactions. The experiments on these animals also demonstrated the other phenomenon of phagocytosis, etc., encountered in the experiments described in the previous series.

The individual protocols are not further detailed here, the reults of the experiments having been fully described in general in the text. (See p. 262.)

XI. THE ANTI-INFECTIOUS POWER OF PLAGUE SERUM.

Since the report of Yersin, Calmette and Borrel in 1895,[88] practically all observers have recognized what may be termed the anti-infectious action of plague immune serum. The disputed point has been rather in regard to the extent of its curative value. As has already been mentioned, it was considered advisable to investigate to what amount these anti-infectious substances became developed in the blood of animals which had been immunized to plague infection by vaccination, and in others immunized in the same manner and the immunity of which to plague infection had in addition been tested, in order to ascertain whether or not these substances could be demonstrated in the blood of such animals in sufficient quantities for the method of their detection to be used as a test of the degree of immunity obtained in the serum of human beings vaccinated against plague. The method employed in investigating this question was as follows: A rat was inoculated intraperitoneally or subcutaneously with the serum to be tested while at the same time the animal was infected by thrusting beneath the skin near the tail a syringe needle which had been dipped in a suspension of the virulent pest organism. Numerous control animals were inoculated in all the series. Rats were used rather than mice in these experiments, since a much more accurate determination of the value of plague serum can be made with these animals than with mice. However, even with rats there is considerable individual variation in regard to their reaction to plague immune serum. Sometimes, after infection, one rat will be saved by a dose of serum, whereas another one of the same size and which has received the same amount of it will succumb. For this reason it is difficult and sometimes impossible accurately to determine the dose of serum which is just sufficient to save the animals. The method used in testing the protection obtained by the rats from the serum inoculations was somewhat more severe than that which has been used by other observers, the infecting needle employed in my experiments being larger; however, in spite of this, it is recognized that the values of the immune sera which I have experimented with were not high; a fact which may be seen from a study of the tables.

The details of the experiments are given in Series 8, 10, 13, 14, 15, 19, 20, 22, 27, 45, 53, and 57 (pp. 274 to 288).

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[88] *Ann. d. l'inst. Pasteur* (1895), **9**, 589.

We learn from a study of these experiments that the anti-infectious substances also become developed very slowly and in very small quantities in animals immunized against pest. While rabbits which have been given a single, small, intravenous inoculation of either living or killed cholera or typhoid bacilli will develop a serum which in high dilutions is protective for guinea pigs against multiple lethal doses of these organisms, on the other hand, rabbits which have been intravenously inoculated with large amounts of killed virulent or with living, attenuated plague cultures, yield sera which, when tested on rats show apparently no protective power whatever against plague infection. Likewise, as large an amount as 5 cubic centimeters of a serum obtained from a rabbit previously inoculated intravenously with 20 milligrams of artificial plague aggressin proved to possess no anti-infectious power when tested on rats. Monkeys which had been immunized against pest by vaccination, or otherwise by inoculation, and which had been shown to be thoroughly immune by the subcutaneous inoculation of multiple lethal doses of the virulent pest strain, furnished sera which also showed no traces of anti-infectious power when tested on rats. Only in the case of one monkey (number 1357) could a slight anti-infectious power be noted and this animal had received repeated, increasing doses of virulent pest bacilli until it resisted the injection of 1½ agar slant cultures of the virulent pest strain. (See Series 57, p. 288.) This series of experiments is particularly important because it illustrates that the negative results in the demonstration of an anti-infectious action obtained with the serum of monkeys less highly immunized against pest, could not be ascribed to the lack of a suitable complement to complete the reaction in the body of the rat.

The sera of thirty-three human beings who had been vaccinated against pest by the inoculation of attenuated cultures were also tested, but in no instance did they show any demonstrable anti-infectious value. However, since animals which had proved themselves, thoroughly immune to pest infection also furnished sera which conferred no greater protection, it would not be reasonable to expect that the human sera would reach a higher power; moreover, the serum of a convalescent plague patient collected five days after the symptoms of the disease had subsided, also showed no anti-infectious value. Only in the case of horse's serum, where the animal had finally been inoculated with repeated, large doses of living pest bacilli, could any marked anti-infectious action be demonstrated and indeed with some of these sera it required as much as 1 cubic centimeter to save the rat from fatal pest infection. Therefore, it is unnecessary to emphasize further that the absence of the anti-infectious substances against pest in sufficient quantities to be demonstrated in a serum can not necessarily be regarded as an evidence of the absence of even a considerable immunity against this disease in the individual in question, since in animals and human beings known to be thoroughly immune to pest infection it has also not been possible to demonstrate these protective substances.

EXPERIMENTS IN RATS DEMONSTRATING THE ANTI-INFECTIOUS POWER OF PLAGUE IMMUNE SERA.

SERIES: 8.—*Experiments with the species "Mus decumanus."*

One 48-hour agar slant culture of "Pest Virulent" (from guinea pig number 1221, second transplant) was suspended in 5 cubic centimeters saline solution. A 5 cubic centimeter needle was dipped in the suspension and thrust under the skin near the root of the tail and then withdrawn. Animals numbered 1252, 1254, and 1256 received pest immune serum intravenously just before being stuck with the infected needle.

Animal No.	Serum.	Result.	Autopsy and remarks.
1249	----------	Dead after 4 days.	No pest bacilli found in smears from heart's blood. Innumerable from region near point of inoculation. Bubonic abscess.
1250	----------	Dead after 5½ days.	Few pest bacilli in heart's blood and in tissues near point of inoculation. Quite numerous in smears from spleen.
1251	----------	Dead after 3½ days.	Numerous pest bacilli in heart's blood.
1252	1.25 cc. antipest serum intraperitoneally.	Alive----------	
1253	----------	Dead after 3½ days.	Fair numbers of pest bacilli in heart's blood.
1254	1 cc. antipest serum intraperitoneally.	Alive----------	Animal very sick for 7 days after inoculation.
1255	----------	Dead after 3½ days.	Pest septicæmia. Fair numbers of pest bacilli in heart's blood.
1256	1 cc. antipest serum intraperitoneally.	Alive----------	
1257	----------	Dead after 3 days.	Smears and cultures from heart and spleen show few pest bacilli. On examining the tissues near the point of inoculation innumerable pest bacilli are found. Animal evidently died of pest intoxication.
1258	----------	Dead after 7 days.	Bubo of the right inguinal gland in which pest bacilli are present. No organisms found in smears from the heart's blood.
1259	----------	Dead after 5 days.	Countless pest bacilli in smears from the tissues near point of inoculation. None seen in smears from the heart's blood.
1260	----------	Dead after 2 days.	No autopsy.

SERIES 10.—*Experiments with rats.*

One 48-hour agar slant culture of "Pest Virulent" (from guinea pig number 1221, second transplant) was suspended in 5 cubic centimeters of saline solution. A 5 cubic centimeter syringe needle was dipped in this suspension and thrust beneath the skin near the root of the tail of each animal. Animals numbered 1265, 1267, 1268, and 1273 received intraperitoneally, just before being stuck with the infected needle, either human serum from cases previously vaccinated against plague, or horses' pest immune serum.

Animal No.	Serum.	Result.	Autopsy and remarks.
1264	------------------------	Dead after 3½ days.	Few pest bacilli in heart's blood.
1265	3 cc. human serum case III.	Dead after 6½ days.	Numerous pest bacilli in smears from spleen and liver.
1266	------------------------	Dead after 5 days.	Left inguinal bubo. Smears from it show numerous pest bacilli and a few cocci.
1267	2 cc. serum mixture human cases VI and VIII.	-----do--------	No organisms found in smears from blood, spleen, near point of inoculation, or right axillary gland. Cultures from the heart and spleen developed a few colonies of pest bacilli.
1268	3 cc. human serum case V.	Dead after 3½ days.	No bacilli found in the heart's blood. Countless pest bacilli in smears made from tissues near point of inoculation. Many bacilli swollen.
1269	------------------------	-----do--------	Very few pest bacilli in smears from heart's blood. Much more numerous in smears from the flank.
1270	------------------------	Dead after 5 days.	Cover slips from spleen show numerous pest bacilli.
1271	------------------------	Dead after 3½ days.	Pest septicæmia. Fair numbers of pest bacilli in smears from heart's blood.
1272	------------------------	-----do--------	Fair numbers of pest bacilli in smears from heart's blood.
1273	2.5 cc. antipest serum.	Dead after 11 days.	Pest septicæmia. Fair numbers of pest bacilli in smears from spleen. Inguinal buboes with countless pest bacilli.
1274	------------------------	Dead after 3 days.	Few pest bacilli in smears from heart's blood.
1275	------------------------	Dead after 22 days.	Chronic pest. Buboes about point of inoculation contained pest bacilli. Numerous organisms in smears from spleen.

SERIES 13.—*Experiments with rats.*

One 48-hour agar slant culture "Pest Virulent" (from monkey number 1282, first transplant) was suspended in 5 cubic centimeters bouillon; a 5 cubic centimeter syringe needle was dipped in this suspension and then thrust beneath the skin of each animal near the root of the tail. Rats numbered 1308, 1310, 1312, 1314, 1316, 1318, and 1320 received intraperitoneally, just prior to being stuck with the infected needle, serum from human cases or from animals which had previously been vaccinated or otherwise inoculated against plague.

SERIES 13—Continued.

Animal No.	Serum intraperineally.	How inoculated.	Result.	Autopsy and remarks.
1307		Stuck with 5 cc. syringe needle.	Dead after 3 days.	Numerous pest bacilli in the spleen.
1308	2 cc. serum from case 10.	do	do	Fair numbers of pest bacilli in smears from spleen.
1309		do	Dead after 5 days.	Innumerable pest bacilli in smears from spleen.
1310	2 cc. serum from case 12.	do	Dead after 3 days.	Smears from spleen show a few bacilli. Organisms very numerous near point of inoculation.
1311		do	do	Few pest bacilli in smears from spleen.
1312	2 cc. of serum from animal 1965.*	do	Dead after 2½ days.	Pest septicæmia. In smears from spleen numerous pest bacilli.
1313		do	do	Numerous pest bacilli in smears from spleen.
1314	2 cc. serum from animal No. No. 1960.†	do	Dead after 3 days.	Numerous pest bacilli in smears from spleen. Many within leucocytes.
1315		do	Dead after 1½ days.	Few pest bacilli in tissues about point of inoculation.
1316	2 cc. serum from animal No. 1960.	do	Dead after 3 days.	Numerous pest bacilli and post-mortem invading bacilli in smears from spleen.
1317		do	do	Pest septicæmia. Innumerable pest bacilli in smears from the spleen.
1318	2 cc. serum from animal No. 1960.	do	do	Many post-mortem invading bacilli and a few pest bacilli in smears from the spleen.
1319		do	Dead after 8 days.	Fair number of pest bacilli in smears from spleen, also a number of large post-mortem invading bacilli present.
1320	2 cc. from animal No. 1965.	do	Dead after 3 days.	Numerous pest bacilli in smears from the spleen.
1321		do	Dead after 15 days.	A few pest bacilli in smears from the spleen. None found near point of inoculation.

*1965 had been previously inoculated intravenously with 5 cubic centimeters Haffkine's prophylactic.

†1960 had been previously inoculated intravenously with one 48-hour culture "Pest Avirulent."

SERIES 14.—*Experiments with rats.*

A 3-day agar culture of "Pest Virulent" (first transplant) was suspended in 5 cubic centimeters of bouillon. A 5 cubic centimeter needle was dipped in this suspension and rats numbered 1330 to 1341 inoculated by thrusting the needle subcutaneously near the root of the tail. Rats numbered 1330 to 1337 received intraperitoneally, just prior to being stuck with the infected needle, serum from human cases which had previously been vaccinated against plague.

Animal No.	Serum intraperitoneally.	How inoculated.	Result.	Autopsy and Remarks.
1330	4 cc. from case 11.	Stuck with 5 cc. syringe needle.	Dead after 3 days.	Fair number of pest bacilli in smears from spleen. Abdominal cavity appears normal.
1331	5 cc. from case 12.	do	do	Only one pest bacillus found in a smear from the spleen. Countless numbers near the point of inoculation. Œdema, hæmorrhagic infiltration, etc., about this area. Abdominal cavity normal in appearance.
1332	5 cc. from case 9.	do	Dead after 5 days.	Very numerous pest bacilli in smears from the spleen.
1333	do	do	Dead after 3 days.	Innumerable pest bacilli in smears from the spleen. Abdominal cavity normal.
1334	2.5 cc. from case 17.	do	do	Innumerable pest bacilli in smears from spleen.
1335	do	do	Dead after 1½ days.	Abdominal cavity appears normal. No serum present. Fair numbers of pest bacilli in smears made from tissues near point of inoculation.
1336	2.5 cc. mixture from cases 18 and 10.	do	Dead after 3 days.	Fair number of pest bacilli in smears from spleen.
1337	do	do	Dead after 4 days.	Few bacilli in smears from spleen. Innumerable about point of inoculation. Infiltration and hæmorrhage about this area.
1338	----------	do	Dead after 3 days.	Fair number of pest bacilli in smears from spleen.
1339	----------	do	Dead after 4 days.	Very numerous pest bacilli in smears from spleen.
1340	----------	do	Dead after 1½ days.	No organisms in smears from spleen. Fair numbers near point of inoculation.
1341	----------	do	do	Fair numbers of pest bacilli about point of inoculation.

SERIES 15.—*Experiments with rats.*

One 48-hour agar culture "Pest Virulent" (guinea pig number 1296) suspended in 5 cubic centimeters bouillon; a 5 cubic centimter needle was dipped in this suspension and then thrust beneath the skin near the root of the tail of each animal. All the rats except the two numbered 1348 and 1353 received intraperitoneally, just prior to being stuck with the infected needle, serum from human cases or animals previously vaccinated against plague.

Animal No.	Serum intraperitoneally.	How inoculated.	Result.	Autopsy and remarks.
1342	5 cc. of serum from case 14.	Stuck with 5 cc. needle.	Dead after 4 days.	Fair number of pest bacilli in smears from spleen.
1343	2 cc. of serum from case 14.	-----do--------	-----do------	Do.
1344	2.5 cc. serum from case 15.	-----do--------	Dead after 2 days.	Numerous pest bacilli and post-mortem invading bacilli in smears from spleen.
1345	-----do------------	-----do--------	Dead after 5 days.	Numerous pest bacilli in smears from spleen.
1346	5 cc. of serum from animal No. 1965 *	-----do--------	Dead after 3 days.	No bacilli found in smears from spleen. Innumerable from tissues near point of inoculation.
1347	-----do------------	-----do--------	-----do------	Very few bacilli found in smears from spleen. Innumerable near point of inoculation.
1348	-------------------	-----do--------	-----do------	Innumerable pest bacilli in smears from the spleen.
1349	5 cc. serum from animal No. 1967. †	-----do--------	Dead after 4 days.	A fair number of pest bacilli in smears from spleen.
1350	-----do------------	-----do--------	Dead after 2 days.	No pest bacilli seen in smears from spleen. A few post-mortem invading bacilli present.
1351	2.5 cc. serum from animal No. 1967.	-----do--------	Dead after 3 days.	A few pest bacilli in smears from spleen.
1352	-----do------------	-----do--------	Dead after 4 days.	Fair number of pest bacilli in smears from spleen. Numerous post-mortem invading bacilli.
1353	-------------------	-----do--------	Dead after 3 days.	Few pest bacilli in smears from spleen.

*Number 1965 had been previously inoculated intravenously with 5 cubic centimeters Haffkine's prophylactic.

†Number 1967 had been previously inoculated intravenously with 1 cubic centimeter autolyzed product digested from 20 milligrams of the strain "Pest Virulent."

SERIES 19.—*Experiments with rats.*

November 9, one 48-hour agar slant culture of "Pest Virulent" (from guinea pig number 1356, first transplant) was suspended in 5 cubic centimeters of bouillon; a 5 cubic centimeter syringe needle was dipped in this suspension and each animal inoculated by thrusting the needle beneath the skin near the root of the tail. Animals numbered 1386 to 1407 received intraperitoneally, just prior to being stuck with the infected needle, serum from human cases previously vaccinated against plague. Rat number 1412 received 0.5 cubic centimeters of an antipestic immune serum.

Animal No.	Serum intraperitoneally.	How inoculated.	Result.	Autopsy and remarks.
1386	4.25 cc. from case 19.	Stuck with infected needle.	Dead after 3 days.	Very numerous pest bacilli in smears from spleen.
1387	do	do	do	Do.
1388	4 cc. from case 20.	do	do	Do.
1389	do	do	Dead after 4 days.	Numerous pest bacilli in smears from spleen.
1390	2 cc. from case 21.	do	do	Numerous pest bacilli in smears from spleen. Intestinal wall dark and hæmorrhagic in places.
1391	3.5 cc. from case 23.	do	do	Innumerable pest bacilli in smears from spleen.
1392	do	do	do	Intestine hæmorrhagic in places. Innumerable pest bacilli in smears from spleen.
1393	3 cc. from case 24.	do	do	Innumerable pest bacilli in smears from spleen.
1394	do	do	do	Very few pest bacilli in smears from the spleen. Innumerable near the point of inoculation.
1395	3 cc. from case 25.	do	do	A few pest bacilli in smears from the spleen.
1396	4 cc. from case 26.	do	do	Numerous pest bacilli in smears from the spleen.
1397	do	do	Dead after 3 days.	A very few typical pest bacilli in smears from spleen.
1398	4.5 cc. from case 27.	do	do	A fair number of pest bacilli in smears from the spleen, appearing in clumps.
1399	do	do	Dead after 4 days.	Numerous pest and post-mortem invading bacilli in smears from the spleen.
1400	5 cc. from case 28.	do	Dead after 8 days.	No bacilli found in smears from the spleen or near point of inoculation.

SERIES 19—Continued.

Animal No.	Serum intraperitoneally.	How inoculated.	Result.	Autopsy and remarks.
1401	5 cc. from case 28.	Stuck with infected needle.	Dead after 7 days.	No pest bacilli found in smears from the spleen or in tissues near the point of inoculation. Spleen small. No infiltration about point of inoculation. It is not clear that this rat died of pest infection.
1402	4 cc. from case 29.	-----do------	Dead after 4 days.	Innumerable pest bacilli in smears from spleen.
1403	-----do------	-----do------	Dead after 3 days.	Very numerous pest bacilli in smears from spleen.
1404	2.5 cc. from case 31.	-----do------	-----do------	Very few pest bacilli in smears from the spleen. Innumerable in smears from tissues near point of inoculation.
1405	-----do------	-----do------	Dead day of inoculation.	
1406	3.25 cc. from case 33.	-----do------	Dead after 3 days.	Very numerous pest bacilli in smears from spleen.
1407	-----do------	-----do------	Dead after 4 days.	Numerous pest bacilli in smears from spleen.
1408	--------------	-----do------	Dead after 2 days.	A few pest bacilli in smears from spleen.
1409	--------------	-----do------	Dead after 4 days.	A fair number of typical pest bacilli in smears from spleen.
1410	--------------	-----do------	Dead after 6 days.	Very numerous pest bacilli in smears from spleen.
1411	--------------	-----do------	Dead after 4 days.	Do.
1412	0.5 cc. antipestic serum.	-----do------	-----do------	Numerous pest bacilli in smears from the spleen. Many swollen. A few near the point of inoculation.
1413	--------------	-----do------	Dead after 3 days.	Very numerous pest bacilli in smears from spleen.

It might be argued that not a sufficient number of animals were inoculated in the different series with each serum for the results to be conclusive, since it is well known that in a series of rats inoculated with a pest immune serum of high value, a certain number of animals are not protected and always die of pest infection after inoculation with a virulent organism, and indeed, the single animal of this series inoculated with the horse's pest immune serum succumbed to pest. However, it seems probable that in such a long series of animals, if there were any the blood of which possessed marked anti-infectious power, the evidences of this fact would be presented by at least some of the animals. Only in the serum of case 28 is there any evidence whatever of an anti-infectious power and there is no further evidence of this nature in any of the other series of rats which have been inoculated with serum from human cases vaccinated against plague. (Compare with Series 22, p. 281 animals 1454 and 1455.)

SERIES 20.—*Experiments with rats.*

November 11, one 70-hour agar slant culture of "Pest Virulent" (from guinea pig number 1354, first transplant), was suspended in 5 cubic centimeters of bouillon; a 5 cubic centimeter syringe needle was dipped in this suspension and thrust beneath the skin near the root of the tail of each animal. Animals numbered 1423, 1425 and 1427, 1429 and 1431 and 1433 received intraperitoneally 1 to 2 cubic centimeters of an antipest immune serum just prior to being stuck with the infected needle.

Animal No.	Amount of serum intraperitoneally.	How inoculated.	Result.	Autopsy and remarks.
1423	2 cc. antipest serum.	Stuck with 5 cc. syringe needle.	Dead after half hour.	Carbolic acid poisoning.
1414		do	Dead after 4 days.	Few bacilli in smears from spleen. Innumerable near point of inoculation.
1425	2 cc. antipest serum.	do	Dead after 11 days.	No organisms found in smears near point of inoculation. In cultures from spleen no pest bacilli.
1426		do	Dead after 4 days.	Innumerable pest bacilli in smears from tissues near point of inoculation.
1427	2 cc. antipest serum.	do	Alive	
1428		do	Dead after 4 days.	Innumerable pest bacilli in smears from spleen.
1429	2 cc. antipest serum.	do	Dead after ½ hour.	Carbolic acid poisoning
1430		do	Dead after 5 days.	Very numerous pest bacilli in smears from spleen.
1431	1 cc. antipest serum.	do	Alive	
1432		do	Dead after 3 days.	Numerous pest bacilli in smears from the spleen.
1433	0.5 cc. antipest serum.	do	Dead after 1 day.	Probably carbolic poisoning.
1434		do	Dead after 3 days.	Numerous pest bacilli in smears from the spleen.

SERIES 22.—*Experiments with rats.*

On November 17, one 48-hour agar slant culture of "Pest Virulent" (from guinea pig number 1383, second transplant) was suspended in 5 cubic centimeters bouillon, a 5 cubic centimeter needle was dipped in this suspension and thrust beneath the skin near the root of the tail of each animal. Rats numbered 1453 to 1463 inclusive received intraperitoneally, just prior to being stuck with the infected needle, serum of monkeys which had been previously inoculated and subsequently had been shown to be entirely immune to large doses of the strain "Pest Virulent."

SERIES 22—Continued.

Animal No.	Serum intraperitoneally.	How infected.	Result.	Autopsy and remarks.
1453	2 cc. serum from monkey No. 1285.*	Stuck with infected needle.	Dead after 4 days.	Very few pest bacilli in smears from point of inoculation.
1454	2 cc. serum from monkey No. 1278.†	do	Dead after 12 days.	Few pest bacilli in smears from spleen. None found at point of inoculation.
1455	do	do	Dead after 6 days.	Numerous pest bacilli in smears from spleen.
1456	do	do	do	Numerous pest bacilli near point of inoculation. None seen in smears from the spleen.
1457	do	do	Dead after 4 days.	Very few bacilli in smears from spleen. No local lesion apparent.
1458	0.75 cc. serum from monkey No. 1278.	do	Dead after 5 days.	Few bacilli in smears from spleen. Spleen greatly swollen.
1459	2 cc. serum from monkey No. 1231.‡	do	Dead after 6 days.	No pest bacilli found in smears from the spleen. Much necrosis and œdema of tissue near point of inoculation.
1460	do	do	Dead after 5 days.	Very numerous pest bacilli in smears from the spleen.
1461	do	do	Dead after 4 days.	Numerous pest bacilli in smears from spleen.
1462	do	do	Dead after 1 day.	
1463	do	do	Dead after 5 days.	Very numerous pest bacilli in smears from spleen.
1464		do	Dead after 4 days.	Do.
1465		do	do	Numerous pest bacilli in smears from spleen.
1466		do	Dead after 2 days.	Few pest bacilli near the local lesion.
1467		do	Dead after 3 days.	Innumerable pest bacilli in smears from spleen.
1468		do	Dead after 5 days.	A few pest bacilli in smears from the spleen.
1469		do	Dead after 4 days.	Fairly numerous pest bacilli in smears from spleen.
1470		do	Dead after 3 days.	Fair number pest bacilli in smears from spleen.

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*Monkey number 1285 was inoculated October 20 by thrusting beneath the skin a 5 cubic centimeter syringe needle which had been dipped in a bouillon suspension of an agar slant of "Pest Virulent." It suffered a mild pest infection and on October 25 was reinoculated with 2 oesen of the strain "Pest Virulent." It survived this inoculation and on November 16 was killed by bleeding and the value of its serum tested as above. Its agglutinative power tested November 17 was found to be entirely negative even in dilutions of 1 or 2.

†Monkey number 1278 was vaccinated October 19 with a culture of "Pest Avirulent." On October 30 it was reinoculated with 2 oesen "Pest Virulent," and proved to be immune. It was killed November 16 by bleeding and the anti-infectious value of its serum tested as above. Its agglutinative power tested November 17 was entirely negative in dilutions as low as 1 to 2 or 1 to 4 after four hours.

‡Monkey number 1231 was inoculated on October 20 by thrusting beneath the skin a 5 cubic centimeter syringe needle that had previously been dipped in a suspension of an agar slant of "Pest Virulent." It suffered a mild pest infection and on October 25 was inoculated with 2 oesen of "Pest Virulent" which it survived. On November 16 it was killed by bleeding and the anti-infectious power of its serum tested as above. Its agglutinative value with the strain "Pest Virulent" was tested on November 17 and found to be entirely negative in dilutions of 1 to 2 and 1 to 4 after four hours.

SERIES 27.—*Experiments with rats.*

One 48-hour agar slant culture of "Pest Virulent" from guinea pig numbered 1668, first transplant), was suspended in 5 cubic centimeters of bouillon; a 5 cubic centimeter syringe needle was dipped in this suspension and thrust beneath the skin near the root of the tail of each animal. Rats numbered 1677 to 1686 received intraperitoneally pest immune serum just before being stuck with the infected needle.

Animal No.	Inoculated intraperitoneally with—	How infected.	Result.	Autopsy and remarks.
1677	1 cc. pest immune serum.	Stuck with infected needle.	Dead after 7 days.	Very numerous pest bacilli and post-mortem organisms in smears from spleen.
1678	do	do	Alive and well.	
1679	do	do	Dead after 4 days.	Innumerable pest bacilli in smears from spleen.
1680	do	do	Dead January 31, after 12 days.	Do.
1681	do	do	Alive and well.	
1682	0.5 cc. pest immune serum.	do	Dead January 22, after 3 days.	Innumerable typical pest bacilli from point of inoculation. Few if any organisms in smears from the spleen.
1683	do	do	Dead after 8 days.	Fair numbers of typical pest bacilli in smears from the spleen.
1684	do	do	Dead after 4 days.	Do.
1685	do	do	Dead after 6 days.	Innumerable pest bacilli in smears from spleen.
1686	do	do	Dead after 3 days.	Numerous pest bacilli in smears from spleen.
1687		do	Dead after 6 days.	Very numerous pest bacilli in smears from spleen.
1688		do	Dead after 3 days.	No organisms found in smears from spleen. Innumerable pest bacilli near point of inoculation.
1689		do	do	Innumerable pest bacilli in smears from spleen.
1690		do	do	Fair numbers of typical pest bacilli in smears from spleen.
1691		do	Dead after 17 days.	Typical pest bacilli from the local lesion. Fair numbers of typical organisms in smears from the spleen.
1692		do	Dead after 4 days.	Numerous typical pest bacilli in smears from spleen.
1693		do	Dead after 3 days.	Numerous pest bacilli in smears from spleen.
1694		do	do	Very numerous typical pest bacilli in smears from the spleen.
1695		do	Dead after 4 days.	Innumerable pest bacilli in smears from the spleen.
1696		do	do	Do.

SERIES 45.—*Experiments with white rats.*

October 26, one 48-hour agar culture of "Pest Virulent" (from guinea pig number 2583, second transplant) was suspended in 5 cubic centimeters of bouillon. A 5 cubic centimeter syringe needle was dipped in this suspension and thrust beneath the skin of the animal near the base of the tail. Ten of the rats received intraperitoneally 1 cubic centimeter of pest immune serum at the time of being stuck with the infected needle, and ten received the same amount of serum twenty-four hours after the infection. Three received, at the time of the infection, human serum from a case which had suffered from bubonic plague and recovered. The blood was collected five days after the symptoms of the disease had subsided and the serum separated and preserved in 0.5 per cent carbolic acid. The remaining 15 rats received no serum.

Animal No.	Serum intraperitoneally.	Infected October 26.	Result.	Autopsy and remarks.
2595	Oct. 26, 1 cc. pest immune serum.	Stuck with 5 cc. syringe-needle dipped in suspension "Pest Virulent."	Dead Oct. 30, after 4 days.	No pest bacilli in smears from spleen. Innumerable about point of inoculation.
2596	Oct. 27, 1 cc. pest immune serum.	do	Dead Oct. 31, after 5 days.	Very few bacilli in smears from spleen. Numerous in tissues near point of inoculation.
2597		do	Dead Oct. 29, after 3 days.	Innumerable pest bacilli near point of inoculation.
2598	Oct. 26, 1 cc. pest immune serum.	do	Dead Nov. 3, after 8 days.	Very few bacilli in smears from spleen. Hæmorrhagic area at point of inoculation, containing numerous bacilli.
2599	Oct. 27, 1 cc. pest immune serum.	do	Dead Oct. 30, after 4 days.	Fair number of pest bacilli in smears from the spleen.
2600		do	do	Very numerous pest bacilli in smears from spleen.
2601	Oct. 26, 1 cc. pest immune serum.	do	Alive and well.	
2602	Oct. 27, 1 cc. pest immune serum.	do	do	
2603		do	Dead Oct. 30, after 4 days.	Numerous pest bacilli in smears from spleen.
2604	Oct. 26, 1 cc. pest immune serum.	do	Alive and well.	
2605	Oct. 27, 1 cc. pest immune serum.	do	Dead Oct. 31, after 5 days.	Fair number of pest bacilli in smears from spleen.
2606		do	Dead Oct. 29, after 3 days.	Do.
2607	Oct. 26, 1 cc. pest immune serum.	do	Dead Nov. 1, after 6 days.	Few pest bacilli in smears from spleen. More numerous near point of inoculation.
2608	Oct. 27, 1 cc. pest immune serum.	do	Alive and well.	
2609		do	do	
2610	Oct. 26, 1 cc. pest immune serum.	do	do	

SERIES 45—Continued.

Animal No.	Serum intraperitoneally.	Infected October 26.	Result.	Autopsy and remarks.
2611	Oct. 27, 1 cc. pest immune serum.	Stuck with 5 cc. syringe-needle dipped in suspension "Pest Virulent."	Alive and well.	
2612		do	Dead Oct. 29, after 3 days.	Innumerable pest bacilli in smears from point of inoculation.
2613	Oct. 26, 1 cc. pest immune serum.	do	Alive and well.	
2614	Oct. 27, 1 cc. pest immune serum.	do	Dead Oct. 29, after 3 days.	Fair number of pest bacilli in smears from spleen.
2615		do	do	Moderate numbers of pest bacilli in smears near point of inoculation.
2616	Oct. 26, 1 cc. pest immune serum.	do	Dead Nov. 2, after 7 days.	Few pest bacilli in smears from the spleen. About point of inoculation a red spot about 3 mm. in diameter. Smears from this area show numerous pest bacilli.
2617	Oct. 27, 1 cc. pest immune serum.	do	Alive and well.	
2618		do	Dead Oct. 30, after 4 days.	Numerous pest bacilli in smears from spleen.
2619	Oct. 26, 1 cc. pest immune serum.	do	Alive and well.	
2620	Oct. 27, 1 cc. pest immune serum.	do	Dead Nov. 2, after 7 days.	Few swollen pest bacilli in smears from spleen. Fairly numerous about point of inoculation, where cocci are also present.
2621		do	Dead Oct. 30, after 4 days.	Numerous pest bacilli in smears from spleen.
2622	Oct. 26, 1 cc. pest immune serum.	do	Alive and well.	
2623	Oct. 27, 1 cc. pest immune serum.	do	Dead Oct. 30, after 4 days.	Few pest bacilli in smears from spleen. Numerous from point of inoculation.
2624		do	Dead Oct. 29, after 3 days.	Innumerable pest bacilli in smears from spleen.
2625	Oct. 26, 2 cc. human serum.	do	do	Very numerous pest bacilli in smears from spleen.
2626	Oct. 26, 1 cc. human serum.	do	do	Fair number of pest bacilli in smears from spleen.
2627		do	do	Innumerable pest bacilli in smears from spleen.
2628		do	Dead Oct. 30, after 4 days.	Few pest bacilli in smears from spleen.
2629		do	Dead Oct. 29, after 3 days.	Numerous pest bacilli in smears from spleen.
2630		do	Dead Oct. 31, after 5 days.	Do.
2631	Oct 26, 1 cc. human serum	do	Dead Oct. 29, after 3 days.	Do.
2632		do	do	Do.

SERIES 53.—*Experiments with white rats.*

November 6, one 3-day culture of "Pest Virulent" from guinea pig number 2594, second transplant, was suspended in 5 cubic centimeters bouillon. A 5 cubic centimeter syringe needle was dipped in this suspension and each rat inoculated by thrusting the needle beneath the skin near the root of the tail. Ten of the rats were given subcutaneously 1 cubic centimeter of a pest immune serum at the time of the infection with the needle. Ten others were given the same amount of serum twenty-four hours after the infection, and those which remained alive of the third ten (nine) were inoculated with 1 cubic centimeter of the serum forty-eight hours after the infection. Ten others were given subcutaneously, at the same time as the others, 1 cubic centimeter of this pest immune serum, which however had been treated previously with the virulent pest strain as described on page 261.

Animal No.	Infected November 6.	Immune serum, 1 cc. subcutaneously on—	Result.	Autopsy and remarks.
2768	Stuck with 5 cc. syringe needle.	Nov. 6	Alive and well.	
2769	do	Nov. 7	Dead Nov. 13, after 7 days.	No pest bacilli found in smears from spleen. One or two swollen organisms found after prolonged search in smears from tissues near point of inoculation.
2770	do	Nov. 8	Alive and well.	
2771	do	Nov. 6	do	
2772	do	Nov. 7	Dead Nov. 9, after 3 days.	Fairly numerous pest bacilli in smears from spleen.
2773	do	Nov. 8	Dead Nov. 11, after 5 days.	No bacilli in smears from spleen. About point of inoculation hæmorrhagic area in which numerous pest bacilli are present.
2774	do	Nov. 6	Dead Nov. 14, after 8 days.	Smears from spleen show one or two swollen bacilli. Smears from point of inoculation show no organisms. No other evidences of pest infection.
2775	do	Nov. 7	Dead Nov. 11, after 5 days.	Numerous post-mortem bacilli and a few pest bacilli in smears from the spleen.
2776	do	Nov. 8	Dead Nov. 9, after 3 days.	Very numerous pest bacilli in smears from spleen.
2777	do	Nov. 6	Alive and well.	
2778	do	Nov. 7	Dead Nov. 11, after 5 days.	No bacilli in smears from spleen. Very numerous fat point of inoculation.
2779	do	Nov. 8	Dead Nov. 12, after 6 days.	Fairly numerous pest bacilli about point of inoculation. None found in smears from spleen.
2780	do	Nov. 6	Alive and well.	
2781	do	Nov. 7	do	
2782	do	Nov. 8	Dead Nov. 11, after 5 days.	Numerous pest bacilli in smears from the spleen.

SERIES 53.—Continued.

Animal No.	Infected November 6.	Immune serum, 1 cc. subcutaneously on—	Result.	Autopsy and remarks.
2783	Stuck with 5 cc. syringe needle.	Nov. 6	Alive and well.	
2784	do	Nov. 7	do	
2785	do	Nov. 8	do	
2786	do	Nov. 6	do	
2787	do	Nov. 7	do	
2788	do	Nov. 8	do	
2789	do	Nov. 6	do	
2790	do	Nov. 7	do	
2791	do	Nov. 8	Dead Nov. 9, after 3 days.	Very numerous pest bacilli in smears from spleen.
2792	do	Nov. 6	Alive and well.	
2793	do	Nov. 7	do	
2794	do	----------	Dead Nov. 8, after 2 days.	Innumerable pest bacilli in smears from spleen.
2795	do	Nov. 6	Alive and well.	
2796	do	Nov. 7	do	
2797	do	Nov. 8	Dead Nov. 8, after ⅛ hour (serum).	Marked symptoms of carbolic poisoning after inoculation of serum. Animal died few minutes after injection. Numerous pest bacilli in smears from spleen.
CONTROLS.				
2798	Stuck with 5 cc. syringe needle.	----------	Dead Nov. 8, after 2 days.	Innumerable pest bacilli in smears from spleen.
2799	do	----------	Dead Nov. 9, after 3 days.	Do.
2800	do	----------	do	Pest and post-mortem bacilli in smears from spleen.
2801	do	----------	Dead Nov. 10, after 4 days.	Fair number of pest bacilli in smears from spleen.
2802	do	----------	Still alive	
2803	do	----------	Dead Nov. 9, after 3 days.	Few bacilli in smears from spleen. Numerous at point of inoculation.
2804	do	----------	do	Innumerable pest bacilli in smears from spleen.
2805	do	----------	Dead Nov. 10, after 4 days.	Numerous pest bacilli in smears from spleen.
2806	do	----------	do	Do.
2807	do	----------	do	Do.

SERIES 53—Continued.

Animal No.	Infected November 6.	Immune serum after binding of amboceptors.	Result.	Autopsy and remarks.
2810	Stuck with 5 cc. syringe needle.	Nov. 6, 1 cc.	Dead Nov. 10, after 9 days.	Pest bacilli in smears from spleen.
2811	do	do	Dead Nov. 11, after 5 days.	Do.
2812	do	do	do	Do.
2813	do	do	Alive and well.	
2814	do	do	Dead Nov. 13, after 7 days.	Do.
2815	do	do	Dead Nov. 10, after 4 days.	Do.
2816	do	do	Alive and well.	
2817	do	do	do	
2818	do	do	Dead Nov. 11, after 5 days.	Do.
2819	do	do	Dead Nov. 13, after 7 days.	Do.

SERIES 57.—*Experiments with rats.*

February 23, one 48-hour culture of "Pest Virulent" from guinea pig numbered 3079, second transplant, was suspended in 5 cubic centimeters bouillon. A 5 cubic centimeter syringe needle was dipped in this suspension and each rat inoculated by thrusting the needle beneath the skin near the root of the tail. Five of the rats were giving subcutaneously varying amounts of serum from a monkey (numbered 1357 *) highly immunized against plague by repeated inoculations in increasing doses of the strain "Pest Virulent." The other rats which served as controls were given no serum.

Animal No.	Serum intraperitoneally.	How infected.	Result.	Autopsy and remarks.
3087	3 cc. serum from monkey No. 1357.*	Stuck with 5 cc. syringe needle.	Alived and well	
3088	do	do	Dead after 14 days	Few pest bacilli in point of inoculation.
3089	2 cc. of serum from monkey No. 1357.	do	Alive and well	
3090	do	do	Dead after 6 days	Few pest bacilli in smears from spleen.
3091	1 cc. serum from monkey No. 1357.	do	Dead after 4 days	Numerous pest bacilli in smears from spleen.
3095		do	do	Do.
3096		do	Dead after 3 days	Do.
3097		do	Dead after 4 days	Do.

*Monkey number 1357 was inoculated as follows: November 2 infected by sticking with a 5 cubic centimeter syringe needle dipped in a suspension of the strain "Pest Virulent". December 2, one-fifth agar slant culture of Pest "Virulent" subcutaneously. December 14, three-fifths of an agar slant, same strain, subcutaneously. January 10, one agar slant "Pest Virulent," subcutaneously. February 1. one and one-half agar slant cultures, "Pest Virulent" subcutaneously. Bled February 22. Twenty cubic centimeters; serum tested as above.

XII. CURATIVE VALUE OF PLAGUE SERUM.

The opinions of different observers are widely divergent in regard to the action of pest immune serum upon the clinical course of the disease in human beings. Amost all authors agree that in advanced cases of infection the serum is of no value. It is not my purpose here to enter into a complete discussion of this subject. The question has recently been reviewed during the present year (1906) by Dujardin-Beaumetz,[89] by Choksy,[90] and by Terni.[91] In 1905 Bannerman[92] reviewed the question of serum therapy in plague in India and the earlier work has been carefully summarized and considered by Dieudonné.[93] From a study of the cases treated and reported in these articles, together with the results obtained from experimentation with animals in the laboratory, there would appear to be little doubt of the value of the serum treatment of plague, provided that the serum is given early enough in the course of the disease. Up to the present time we have not been able to obtain a serum which shows any demonstrable antitoxic value by any known method of preparation, since indeed we have been unable to obtain a soluble pest toxin. The work of Markl upon this subject has already been considered on page 184 of this article.

Besredka's[94] studies upon the endotoxins of typhoid and pest bacilli may be mentioned in connection with the question, but they have not been elaborated further. Terni[95] thought that he was able to prepare an anti-serum which was especially active against the specific pest toxin, by the inoculation of the animal furnishing the serum with peritoneal exudates from guinea pigs dead of pest and with the serum from pest buboes, etc. (natural plague aggressin). Such a serum he regarded as being better than those sera obtained by the other known methods. However, I have shown (see p. 236) that the immunity obtained by the injection of natural plague aggressin is not of a different nature from that secured by the inoculation of living pest cultures and that, while inoculations of natural plague aggressin produce an immunity greater than that which can be obtained from those of artificial plague aggressin, the

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[89] *Bull. d. l'inst. Pasteur.* (1906), 4, 473.

[90] Report on the Treatment of Plague, Bombay (1906).

[91] *Ztschr. f. Hyg. u. Infectionskrankh.* Leipz. (1906), 54, 386.

[92] *Scient. Mem. Med. Off. India* (1905), 20.

[93] *Handb. d. path. Mik.* (Kolle & Wassermann) (1904), 4, 949.

[94] *Ann. d. l'inst. Pasteur* (1905), 19, 477.

[95] *Loc. cit.*

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inoculation of living cultures of pest bacilli gives rise to a higher immunity than either of the first two methods mentioned.

As I have already explained in discussing the subject of the mechanism of the action of plague immune serum, the serum exerts its immunizing effect primarily by binding through its amboceptors the receptors of the plague bacilli and after this change has taken place, phagocytosis of the microörganisms occurs. The success of the serum treatment in plague would appear to depend particularly upon the number of plague bacilli in the animal organism at the time of the inoculation of the serum; that is, upon the length of time the serum is injected after the infection has occurred. If the organism is already overwhelmed with bacteria at the time of the introduction of the serum, almost no favorable change will be noted in the course of the disease, because the serum is merely anti-infectious and is not anti-toxic. A study of the experiments recorded in Series 45 and 53 (pp. 284 and 286) confirms these views. In Series 45 we see that in ten rats inoculated with immune serum at the time of their infection with pest bacilli, 60 per cent survived and 40 per cent succumbed to the infection, while of ten rats which were inoculated with the serum twenty-four hours *after* the pest infection only 40 per cent survived and 60 per cent died. The experiments recorded in Series 53 are particularly important in this connection. Here the animals were inoculated with the serum in three series: One at the time of the infection, a second twenty-four hours following the infection, and the third forty-eight hours after the infection. The mortality in the first series was 10 per cent, in the second 40 per cent, and in the third 66.6 per cent.

It is true that there is nothing new in the idea that rodents may be protected against a fatal outcome of the pest infection by the injection of plague immune serum which has been introduced at varying periods of time after that of the infection. In fact, as long ago as 1895, Yersin, Calmette and Borrel[96] called attention to the fact that mice which had been infected with pest could be saved from death if an inoculation of 1.5 cubic centimeters of pest immune serum were to be given them as late as twelve hours after the time of the pest infection. Moreover, this method is still used in the Pasteur Institute and elsewhere (usually with rats), for the purpose of testing the curative value of the manufactured pest immune serum.

The German Plague Commission also found it possible to save monkeys which had previously been infected with pest by the inoculation of pest immune serum injected as late as from twelve to twenty-four hours after the time of the infection, and many other observers have recorded numerous similar successful experiments in which rats were employed. I therefore have not called attention to my experiments recorded in Series 51 with the sole object of again illustrating this action of plague immune serum;

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[96] *Ann. d. l'inst. Pasteur* (1895), **9**, 591.

however, this series of experiments serves to illustrate particularly well the relations between the duration of the infection and results of the serum treatment.

For example, in the case of rat number 2797 which succumbed from carbolic acid poisoning one-half hour after the injection of the immune serum, an autopsy performed immediately after death showed that numerous pest bacilli already had invaded the spleen. This animal had been infected with pest on November 6 and the serum was inoculated two days later, November 8. Rats numbered 2794 and 2798, which were also infected on September 6, died on September 8 of pest infection before receiving serum. At autopsy the infection likewise was found to be well advanced in both animals and innumerable bacilli were present in each instance in the spleen.

It therefore seems fair to presume that, in a good proportion of the other rats inoculated on November 6 with the same sized dose, the infection had also already advanced to a somewhat similar stage, and therefore probably in many of these animals the pest bacilli had also already invaded the spleen. Nevertheless, 33.3 per cent of those animals which received the serum forty-eight hours after the time of infection survived. Therefore, these experiments demonstrate not only that animals may be saved by the injection of immune serum at a period subsequent to the incurrence of the pest infection, but that animals in which the disease is fairly well advanced may also be saved by the serum, the percentage of deaths varying directly with the length of the interval between pest infection and serum inoculation.

Choksy, who has a wide experience with the serum treatment of plague, states:

> Much depends upon the early and free use of the serum. In patients injected on the first day or within a few hours of the onset of the symptoms, one injection of 100 cubic centimeters followed by another after 6 to 8 hours and then if necessary by a third after a similar interval, would cut short the attack if the case be not pneumonic, malignant or septicæmic.

He also emphasizes the fact that the earlier the serum is used, the more efficacious will it be and that if good results are to be obtained from serum therapy the patient must be treated on the first day of the illness. He admits that the serum can not favorably influence all types of plague, or even the malignant forms of the bubonic type, but he shows that it is the only treatment capable of saving a large proportion in a certain class of patients. Therefore, it seems that in man there is a narrow limit beyond which the antiplague serum will not act, but that within this limit its use in man, as in animals, is efficacious.

Roux, Yersin, and Dujardin-Beaumetz all emphasize that in the serum treatment of plague, as in that of cholera, the injection of the serum must be given intravenously, on the ground that the absorption from the cellular tissue is very slow and that by the intravenous inoculation the organism may be saturated with the antitoxin immediately and by a smaller dose than it could be if used subcutaneously. Dujardin-Beaumetz [97] further remarks that in cases where the pest bacilli appear early in the general circulation, there are additional advantages for the intravenous inoculation of serum. In my opinion in cases where plague bacilli are already circulating in the blood there is little hope of a successful result with the serum, either in man or animals. The treatment of cholera with intravenous inoculations of the "antitoxic cholera serum" of Brau and Denier so far has not been very successful, apparently because the serum does not evidence sufficient antitoxic action. In plague immune serum also, no matter by what method it is prepared, as has been demonstrated, we are not able to obtain any antitoxic action of value and so, for the same reason, we perhaps may not be justified in the hope of obtaining in plague any different results from the intravenous than from the subcutaneous inoculation of the serum, other than those which, as the French authors have suggested, would result from its more rapid absorption. By the intraperitoneal inoculation, as suggested by Dujardin-Beaumetz and others, a rapid absorption of the serum may also be secured. The cases of plague in which serum therapy will accomplish the best results are those where the infection is still confined to certain of the lymph channels. There is no doubt of the great prophylactic value of plague immune serum but, as was mentioned in Chapter IV, the passive immunity conferred by it disappears after a few days.

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[97] *Loc. cit.*, p. 481.

XIII. VIRULENCE OF THE PLAGUE BACILLUS.

INCREASE OF VIRULENCE OF THE ORGANISM.

The behavior of the pest bacillus in relation to its virulence is very interesting and in many respects quite unusual, when it is compared with that of the majority of the microörganisms giving rise to our other common infectious diseases. The question obviously is also a very important one from an epidemiological standpoint and therefore will be discussed somewhat at length.

In 1895, Yersin, Calmette and Borrel[98] maintained that in a series of passages of the pest bacillus through a given species of animal, an increase of virulence ("a fixed virulence") was obtained for this species of animal only, and that by continued passages through a series of animals of one species, its virulence for other species became reduced. Dujardin-Beaumetz,[99] during the present year also speaks of a hyper-virulence of the pest bacillus obtained by passage through guinea pigs and rats. Hankin[100] found that the virulence of plague bacilli became reduced by passage through rats and that he was not able to kill more than three or four rats in a series, even though the culture in the beginning was fully virulent. On the other hand, he found that the virulence of the organism could be increased by passage from mouse to mouse.

Walton,[101] also observed that a virulent strain of pest became attenuated by successive passages from rat to rat. He used a suspension of a portion of the spleen of the dead animal for infecting the second one, and so on. He also was not able to infect more than three rats in a series by this method. Otto,[102] from numerous carefully performed experiments, concluded first that in the passage of pest bacilli from guinea pig to guinea pig without growth on artificial media, there resulted no dimunition in virulence of the organism either for the guinea pig or for the rabbit, rat or mouse, and second, that it did not appear to be possible by passing the organism successively through guinea pigs to obtain a substantial increase in the virulence of a culture of sufficient pathogenesis at the time of the beginning of the experiment, to cause the death of a guinea pig.

The Plague Commission in India under the direction of Martin and Lamb[103] have also very recently reported upon the effect upon its virulence of the passage of *Bacillus pestis* through series of rats, when the organism was given by subcutaneous inoculation without intermediate culture. Each rat in the series was infected with a suspension of the spleen of the animal which had died previously.

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[98] *Ann. de l'Inst. Pasteur* (1895), **9**, 589.

[99] *Bull. de l'Inst. Pasteur* (1906), **4**, 473.

[100] Rept. of the Indian Plague Commission (1899), **2**, 22.

[101] Rept. of Indian Plague Commission (1900), **3**, 337.

[102] *Zeit. f. Hyg. u. Infectionskrankh.* Leipz. (1902), **41**, 380; (1904), **48**, 430.

[103] *Jour. de Hyg.* (1906), **6**, 496, 502.

It was found possible to carry on the series in this way as far as the twenty-sixth passage, when apparently the experiments were discontinued. The rats were all wild, Bombay rats and were not selected with reference to species. No evidence was obtained by these experiments that the virulence of the bacillus used for the passages underwent any alteration nor was any given by those in which cutaneous inoculation of a series of rats was attempted. Notwithstanding the failure of Otto and of the Indian Plague Commission perceptibly to increase the virulence of pest bacilli by repeated inoculation from guinea pig to guinea pig or from rat to rat, Dieudonné[104] states that pest cultures which have become weakly virulent may again be rendered more infectious by repeated passages through such animals as guinea pigs and rats. He also recommends the passage from the pneumonic lung of the infected animal to the healthy lung of the second animal, such as the rat or ape, and so on through a series as a means of increasing the virulence of this organism.

Balzaroff[105] also found that the virulence of a pest strain could be increased if it was passed successively for several generations from the pneumonic lung of an animal, by nasal infection, to the healthy lung of another one.

An organism which had not the power of killing animals by hypodermic inoculation was said to be restored to virulence by inoculating a guinea pig with it in the nostril and upon the death of this animal, inoculating by the same portal of entry a second one with a portion of the spleen of the first. After the third or fourth passage the organism was said again to have become virulent.

Marsh[106] claimed that if the organism was cultivated at certain temperatures in mixtures of carbonic acid gas and ordinary air, the bacillus increased in virulence and retained its vitality for a long time.

Albrecht and Gohn,[107] and Kolle and Martini[108] also found that by means of repeated animal passages, without growth on artificial media, a strain of the pest bacillus was rendered more virulent for the species of animal employed in the experiment, and also for other species which were markedly susceptible to pest. On the contrary, the transmission of the pest virus from rabbit to rabbit apparently resulted in a reduction of the virulence of the organism.

Kolle and Hetsch[109] also state that some strains of pest bacilli can be rendered more virulent by animal passage.

My experiments, which had the object of increasing the virulence of the pest bacillus, have been carried on in guinea pigs and monkeys and both virulent and avirulent strains of the organism have been used. However, no perceptible increase in virulence has been obtained for either rats, guinea pigs or monkeys. The strain "Pest Virulent," as has already been stated, has been passed successively for over 247 passages from guinea pig to guinea pig without growth on artificial media. (See table of Series 58, p. 296.) These passages have occupied more than a year and a half. The method of infection was carried on as follows:

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[104] *Handb. d. path. Mik.* (Kolle and Wassermann) (1903), **2,** 516.

[105] *Ann. d. l'inst. Pasteur* (1899), **13,** 385.

[106] Rep. of Indian Plague Commission (1898–99), **3,** 73; also (1898–99), **5,** App. III, 480.

[107] Loc. cit.

[108] *Deutsche med. Wchnschr.* (1902), **28,** 1.

[109] Die Exper. Bakt. u. die Infektionskrankh., Berl. and Wien (1906), 214.

The first guinea pig of the series was inoculated subcutaneously with one oese of the strain "Pest Virulent" isolated from a human case of plague. Upon the death of the animal two days later, its spleen was removed and a portion of it rubbed over a shaved area on the abdomen of a healthy guinea pig (the next animal of the series). The skin was sometimes slightly scarified with a knife. This method was employed on the third animal, when the second succumbed, and so on without a break throughout the series. In a few instances the autopsy upon the animal and the removal of the spleen did not take place until nearly forty-eight hours after the death of the animal. In a few others, the spleen was removed shortly after the death of the guinea pig and placed on ice and employed for the next inoculation twenty-four hours later. These instances are all noted in the table. They caused no change in the results of the inoculations.

The accompanying table (58) shows that the virulence of the organism during this whole time has not varied. Moreover, its virulence not only for guinea pigs, but for rats and monkeys as well, has remained the same at the end of the series as it was at the beginning; this may be seen from the numerous control animals inoculated from time to time with this strain throughout the series of animal experiments detailed on pages 187 to 288. A glance at Table 58 shows that moderate differences in susceptibility exist in the different guinea pigs, the most susceptible dying in from two to four days after inoculation and the more resistant usually in from seven to eight days. The animals were not always of the same age and weight and for this reason some slight variation in susceptibility also probably occurred.

Only one animal survived for twelve days and but one other for eleven days after the infection. These animals represent the thirty-sixth and one hundred and twentieth passages respectively. The fact that in the thirty-fifth and thirty-seventh passages, the animals died six and four days respectively after infection, and in the one hundred and nineteenth and one hundred and twenty-first passages, four and three days respectively after inoculation, demonstrates that not even a temporary change in virulence in the organisms had occurred at these periods. The length of time the animals lived during the thirty-sixth and one hundred and twentieth passages therefore was probably due to their natural and slightly increased relative resistance to pest infection. Animal number 198 died twenty-four hours after its inoculation. The animal was doubtless sick at the time of the infection.

Series 58.—*Showing passage of the strain "Pest Virulent" from guinea pig to guinea pig through 247 animals by cutaneous inoculation from the spleen of one animal to the abdomen of the next without growth on artificial media.*

No. of passage.	Animal No.	Date inoculated.	How inoculated.	Date of death.	Number of days lived.	Culture from heart.
1	1220	Oct. 8	1 oese "Pest Virulent" subcutaneously.	Oct. 10	2	*Bacillus pestis.*
2	1221	Oct. 10	Spleen 1220	Oct. 14	4	Do.
3	1243	Oct. 14	Spleen 1221	Oct. 19	5	Do.
4	1276	Oct. 19	Spleen 1243	Oct. 24	5	Do.
5	1297	Oct. 24	Spleen 1276	Oct. 30	6	Do.
6	1323	Oct. 30	Spleen 1297	Nov. 2	2	Do.
7	1324	do	do	do	2	Do.
8	1354	Nov. 2	Spleen 1324	Nov. 7	5	Do.
9	1355	do	do	Nov. 10	8	Do.
10	1356	do	do	Nov. 7	5	Do.
11	1383	Nov. 7	Spleen 1356	Nov. 13	6	Do.
12	1384	do	do	Nov. 14	7	Do.
13	1435	Nov. 13	Spleen 1383	Nov. 16	3	Do.
14	1436	do	do	do	3	Do.
15	1437	Nov. 14	Spleen 1384	Nev. 17	3	Do
16	1451	Nov. 16	Spleen 1436	Nov. 20	4	Do.
17	1452	do	do	Nov. 19	3	Do.
18	1471	Nov. 17	Spleen 1437	Nov. 20	3	Do.
19	1472	Nov. 19	Spleen 1452	Nov. 24	5	Do.
20	1479	Nov. 20	Spleen 1451	do	4	Do.
21	1480	do	Spleen 1471	Nov. 27	7	Do.
22	1495	Nov. 24	Spleen 1479	do	3	Do.
23	1496	do	Spleen 1472	Nov. 30	6	Do.
24	1497	Nov. 27	Spleen 1495	Dec. 3	6	Do.
25	1498	do	Spleen 1480	Dec. 4	7	Do.
26	1514	Nov. 30	Spleen 1496	do	4	Do.
27	1515	Dec. 3	Spleen 1497	Dec. 7	4	Do.
28	1516	Dec. 4	Spleen 1498	Dec. 13	9	Do.
29	1517	do	Spleen 1514	Dec. 10	6	Do.
30	1557	Dec. 7	Spleen 1515	do	3	Do.
31	1579	Dec. 10	Spleen 1557	Dec. 16	6	Do.
32	1580	do	Spleen 1517	Dec. 15	5	Do.
33	1588	Dec. 13	Spleen 1516	Dec. 20	7	Do.
34	1591	Dec. 15	Spleen 1580	Dec. 19	4	Do.
35	1592	Dec. 16	Spleen 1579	Dec. 22	6	Do.
36	1606	Dec. 19	Spleen 1591	Dec. 31	11	Do.
37	1607	Dec. 20	Spleen 1588	Dec. 24	4	Do.
38	1610	Dec. 22	Spleen 1592	Dec. 27	5	Do.
39	1612	Dec. 24	Spleen 1607	Dec. 28	4	Do.
40	1625	Dec. 27	Spleen 1610	Dec. 31	4.	Do.
41	1627	Dec. 28	Spleen 1612	Jan. 3	6	Do.
42	1630	Dec. 31	Spleen 1606	Jan. 4	4	Do.
43	1631	do	Spleen 1625	do	4	Do.
44	1632	Jan. 3	Spleen 1627	Jan. 6	3	Do.
45	1634	Jan. 4	Spleen 1630	Jan. 7	3	Do.
46	1635	do	Spleen 1631	do	3	Do.
47	1643	Jan. 6	Spleen 1632	Jan. 10	4	Do.
48	1645	Jan. 7	Spleen 1634	Jan. 9	2	Do.
49	1646	do	Spleen 1635	do	2	Do.

SERIES 58.—*Showing passage of the strain "Pest Virulent," etc.*—Continued.

No. of passage.	Animal No.	Date inoculated.	How inoculated.	Date of death.	Number of days lived.	Culture from heart.
50	1648	Jan. 9	Spleen 1645	Jan. 12	3	*Bacillus pestis.*
51	1649	do	Spleen 1646	Jan. 14	5	Do.
52	1650	Jan. 10	Spleen 1643	do	4	Do.
53	1653	Jan. 12	Spleen 1648	Jan. 15	3	Do.
54	1667	Jan. 14	Spleen 1649	Jan. 18	4	Do.
55	1668	do	Spleen 1650	Jan. 19	5	Do.
56	1669	Jan. 15	Spleen 1653	Jan. 17	2½	Do.
57	1673	Jan. 17	Spleen 1669	Jan. 21	4	Do.
58	1674	Jan. 18	Spleen 1667	Jan. 22	4	Do.
59	1676	Jan. 19	Spleen 1668	Jan. 23	4	Do.
60	1697	Jan. 22	Spleen 1673*	Jan. 24	2	Do.
61	1698	do	Spleen 1674	do	2	Do.
62	1700	Jan. 23	Spleen 1676	Jan. 25	2	Do.
63	1702	Jan. 24	Spleen 1697	Jan. 26	2	Do.
64	1703	do	Spleen 1698	Jan. 27	3	Do.
65	1704	Jan. 25	Spleen 1700	Jan. 28	3	Do.
66	1708	Jan. 26	Spleen 1702	Jan. 29	3	Do.
67	1709	Jan. 27	Spleen 1703	Feb. 6	10	Do.
68	1711	Jan. 28	Spleen 1704	Feb. 4	7	Do.
69	1712	Jan. 29	Spleen 1708	Jan. 31	3	Do.
70	1714	Jan. 31	Spleen 1712	Feb. 4	4	Do.
71	1724	Feb. 4	Spleen 1711	Feb. 8	4	Do.
72	1725	do	Spleen 1714	Feb. 7	3	Do.
73	1728	Feb. 7	Spleen 1709	Feb. 10	3	Do.
74	1729	do	Spleen 1725	Feb. 12	5	Do.
75	1745	Feb. 8	Spleen 1724	do	4	Do.
76	1752	Feb. 11	Spleen 1728*	Feb. 13	2½	Do.
77	1755	Feb. 12	Spleen 1729	Feb. 15	3	Do.
78	1756	do	Spleen 1745	Feb. 19	7	Do.
79	1759	Feb. 13	Spleen 1752	Feb. 16	3	Do.
80	1761	Feb. 15	Spleen 1755	Feb. 17	2	Do.
81	1762	Feb. 16	Spleen 1759	Feb. 19	4	Do.
82	1764	Feb. 17	Spleen 1761	Feb. 21	4	Do.
83	1765	Feb. 19	Spleen 1756	Feb. 23	4	Do.
84	1766	do	Spleen 1762	Feb. 24	5	Do.
85	1768	Feb. 21	Spleen 1764	Feb. 26	5	Do.
86	1769	Feb. 23	Spleen 1765	Mar. 2	7	Do.
87	1770	Feb. 24	Spleen 1766	Feb. 27	3	Do.
88	1771	do	do	Mar. 1	4	Do.
89	1772	do	do	Mar. 5	9	Do.
90	1798	Feb. 26	Spleen 1768	Mar. 1	4	Do.
91	1799	do	do	do	4	Do.
92	1800	do	do	Mar. 2	5	Do.
93	1818	Feb. 27	Spleen 1770	Mar. 3	4	Do.
94	1819	Mar. 2	Spleen 1800	Mar. 5	3	Do.
95	1820	Mar. 3	Spleen 1818	Mar. 7	4	Do.
96	1821	Mar. 5	Spleen 1819	Mar. 10	5	Do.
97	1831	Mar. 8	Spleen 1820 †	Mar. 12	4	Do.
98	1833	Mar. 10	Spleen 1821	Mar. 13	3	Do.
99	1834	Mar. 12	Spleen 1831	Mar. 16	4	Do.

*Spleen laid on ice one day before inoculation.

† Spleen placed on ice twenty-four hours before inoculation.

SERIES 58.—*Showing passage of the strain "Pest Virulent," etc.*—Continued.

No. of passage.	Animal No.	Date inoculated.	How inoculated.	Date of death.	Number of days lived.	Culture from heart.
100	1835	Mar. 13	Spleen 1833	Mar. 16	3	*Bacillus pestis.*
101	1905	Mar. 16	Spleen 1834	Mar. 19	3	Do.
102	1906	do	Spleen 1835	do	3	Do.
103	1907	Mar. 19	Spleen 1905	Mar. 23	4	Do.
104	1908	do	Spleen 1906	Mar. 21	2	Do.
105	1909	Mar. 21	Spleen 1907	Mar. 26	5	Do.
106	1914	Mar. 23	Spleen 1907	do	3	Do.
107	1928	Mar. 26	Spleen 1909	Mar. 31	5	Do.
108	1929	do	Spleen			Alive.
109	1934	Mar. 31	Spleen 1928	Apr. 3	3	*Bacillus pestis.*
110	1935	Apr. 3	Spleen 1934	Apr. 7	4	Do.
111	1936	Apr. 7	Spleen 1935	Apr. 11	4	Do.
112	1937	do	do	Apr. 10	3	Do.
113	1938	Apr. 10	Spleen 1937	Apr. 14	4	Do.
114	1939	Apr. 11	Spleen 1936	Apr. 16	5	Do.
115	1940	Apr. 14	Spleen 1938	Apr. 17	3	Do.
116	1941	Apr. 16	Spleen 1939	Apr. 22	6	Do.
117	1946	Apr. 17	Spleen 1940	Apr. 20	3	Do.
118	1947	Apr. 20	Spleen 1946	Apr. 24	4	Do.
119	1948	Apr. 23	Spleen 1941*	Apr. 27	4	Do.
120	1952	Apr. 24	Spleen 1947	May 6	12	Do.
121	1953	Apr. 27	Spleen 1948	Apr. 30	3	Do.
122	1954	Apr. 30	Spleen 1953	May 4	4	Do.
123	1956	May 4	Spleen 1954	May 7	3	Do.
124	1957	May 7	Spleen 1952 †	May 13	6	Do.
125	1958	do	Spleen 1956	May 12	5	Do.
126	1959	May 12	Spleen 1958	May 18	6	Do.
127	1962	May 13	Spleen 1957	May 16	3	Do.
128	1997	May 16	Spleen 1962	May 21	5	Do.
129	2005	May 18	Spleen 1959	May 23	5	Do.
130	2052	May 4	Spleen 1997	May 28	7	Do.
131	2055	May 23	Spleen 2005	May 29	6	Do.
132	2060	May 28	Spleen 2052	May 31	3	Do.
133	2061	May 29	Spleen 2055	June 1	3	Do.
134	2062	May 31	Spleen 2060	June 4	4	Do.
135	2065	June 1	Spleen 2060	June 5	4	Do.
136	2066	June 4	Spleen 2062	June 9	5	Do.
137	2067	June 5	Spleen 2065	do	4	Do.
138	2073	June 9	Spleen 2066	June 14	5	Do.
139	2074	do	Spleen 2067	June 13	4	Do.
140	2123	June 13	Spleen 2074	June 19	6	Do.
141	2124	June 14	Spleen 2073	June 18	4	Do.
142	2140	June 18	Spleen 2124	June 25	7	Do.
143	2141	do	do	June 27	9	Do.
144	2142	June 19	Spleen 2123	June 25	6	Do.
145	2143	June 25	Spleen 2140	June 30	5	Do.
146	2144	do	Spleen 2142	June 29	4	Do.
147	2145	June 29	Spleen 2144	July 2	3	Do.
148	2167	July 3	Spleen 2143	July 6	3	Do.
149	2168	July 2	Spleen 2145	July 7	5	Do.

*Animal 1941 died on Apr. 22. Not autopsied until Apr. 23.

†Animal 1952 died May 6. Not autopsied until May 7.

SERIES 58.—*Showing passage of the strain "Pest Virulent," etc.*—Continued.

No. of passage.	Animal No.	Date inoculated.	How inoculated.	Date of death.	Number of days lived.	Culture from heart.
150	2174	July 6	Spleen 2167	July 12	6	*Bacillus pestis*
151	2175	July 7	Spleen 2168	July 11	4	Do.
152	2176	July 11	Spleen 2175	July 17	6	Do.
153	2177	July 12	Spleen 2174	July 16	4	Do.
154	2178	July 16	Spleen 2177	July 23	7	Do.
155	2179	July 17	Spleen 2176	July 20	3	Do.
156	2183	July 20	Spleen 2179	July 24	4	Do.
157	2193	July 23	Spleen 2178	Aug. 3	10	Do.
158	2204	July 24	Spleen 2183	July 31	7	Do.
159	2230	July 31	Spleen 2204	Aug. 6	7	Do.
160	2248	Aug. 3	Spleen 2193	do	3	Do.
161	2271	Aug. 6	Spleen 2248	Aug. 11	5	Do.
162	2272	do	Spleen 2230	Aug. 13	7	Do.
163	2283	Aug. 11	Spleen 2271	Aug. 15	4	Do.
164	2293	Aug. 13	Spleen 2272	Aug. 19	6	Do.
165	2303	Aug. 15	Spleen 2283	Aug. 20	5	Do.
166	2315	Aug. 20	Spleen 2293*	Aug. 24	4	Do.
167	2316	do	Spleen 2303	do	4	Do.
168	2322	Aug. 24	Spleen 2315	Aug. 29	5	Do.
169	2323	do	Spleen 2316	Aug. 31	7	Do.
170	2387	Aug. 29	Spleen 2322	Sept. 4	6	Do.
171	2388	Aug. 31	Spleen 2323	Sept. 3	3	Do.
172	2392	Sept. 3	Spleen 2388	Sept. 7	4	Do.
173	2395	Sept. 4	Spleen 2387	Sept. 10	6	Do.
174	2410	Sept. 7	Spleen 2392	Sept. 12	5	Do.
175	2411	Sept. 10	Spleen 2395	Sept. 15	5	Do.
176	2416	Sept. 12	Spleen 2410	Sept. 14	2	Do.
177	2428	Sept. 14	Spleen 2416	Sept. 17	3	Do.
178	2429	Sept. 15	Spleen 2411	Sept. 21	6	Do.
179	2446	Sept. 17	Spleen 2428	do	4	Do.
180	2481	Sept. 21	Spleen 2429	Sept. 24	3	Do.
181	2482	do	Spleen 2446	Sept. 26	5	Do.
182	2483	Sept. 24	Spleen 2481	Oct. 2	8	Do.
183	2485	Sept. 26	Spleen 2482	Sept. 29	3	Do.
184	2496	do	Spleen 2485	Oct. 2	3	Do.
185	2498	Oct. 2	Spleen 2483	Oct. 5	3	Do.
186	2499	do	Spleen 2496	Oct. 4	2	Do.
187	2500	Oct. 4	Spleen 2499	Oct. 5	4	Do.
188	2501	Oct. 5	Spleen 2498	Oct. 8	3	Do.
189	2502	Oct. 8	Spleen 2500	Oct. 13	5	Do.
190	2503	do	Spleen 2501	Oct. 12	4	Do.
191	2507	Oct. 12	Spleen 2503	Oct. 17	5	Do.
192	2560	Oct. 13	Spleen 2502	Oct. 18	5	Do.
193	2583	Oct. 17	Spleen 2507	Oct. 22	5	Do.
194	2585	Oct. 18	Spleen 2560	Oct. 23	5	Do.
195	2586	Oct. 22	Spleen 2583	Oct. 26	4	Do.
196	2587	Oct. 23	Spleen 2585	do	3	Do.
197	2594	Oct. 26	Spleen 2587	Oct. 30	4	Do.
198	2663	Oct. 26	Spleen 2586	Oct. 27	†1	Do.
199	2694	Oct. 27	Spleen 2663	Nov. 5	9	Do.

*Animal 2293 died Aug. 19. Not autopsied until Aug. 20.

†Probably sick, at time of pest inoculation.

Series 58.—*Showing passage of the strain "Pest Virulent," etc.*—Continued.

No. of passage.	Animal No.	Date inoculated.	How inoculated.	Date of death.	Number of days lived.	Culture from heart.
200	2736	Oct. 30	Spleen 2594	Nov. 2	3	*Bacillus pestis.*
201	2750	Nov. 2	Spleen 2736	Nov. 5	3	Do.
202	2764	Nov. 5	Spleen 2694	Nov. 8	3	Do.
201	2765	do	Spleen 2750	Nov. 9	4	Do.
202	2827	Nov. 8	Spleen 2764	Nov. 14	6	Do.
203	2828	Nov. 9	Spleen 2765	Nov. 11	2	Do.
204	2841	Nov. 11	Spleen 2828	Nov. 14	3	Do.
205	2858	Nov. 14	Spleen 2841	Nov. 22	8	Do.
206	2859	Nov. 15	Spleen 2827	Nov. 19	4	Do.
207	2865	Nov. 19	Spleen 2859	Nov. 23	4	Do.
208	2866	Nov. 22	Spleen 2858	Nov. 26	4	Do.
209	2869	Nov. 23	Spleen 2865	Nov. 28	5	Do.
210	2895	Nov. 26	Spleen 2866	Nov. 30	4	Do.
211	2921	Nov. 28	Spleen 2869	Dec. 3	5	Do.
212	2923	Dec. 3	Spleen 2921	Dec. 8	5	Do.
213	2949	Dec. 8	Spleen 2923	Dec. 11	3	Do.
214	2954	Dec. 11	Spleen 2949	Dec. 15	4	Do.
215	2961	Dec. 15	Spleen 2954	Dec. 18	3	Do.
216	2965	Dec. 18	Spleen 2961	Dec. 22	4	Do.
217	2968	Dec. 22	Spleen 2965	Dec. 26	4	Do.
218	2970	Dec. 26	Spleen 2968	Dec. 30	4	Do.
219	2978	Dec. 30	Spleen 2970	Jan. 5	6	Do.
220	2982	Jan. 5	Spleen 2978	Jan. 8	3	Do.
221	2987	Jan. 8	Spleen 2982	Jan. 11	3	Do.
222	2990	Jan. 11	Spleen 2987	Jan. 15	4	Do.
223	2993	Jan. 15	Spleen 2990	Jan. 17	2	Do.
224	3009	Jan. 17	Spleen 2993	Jan. 19	2	Do.
225	3010	Jan. 19	Spleen 3009	Jan. 23	4	Do.
226	3016	Jan. 23	Spleen 3010	Jan. 28	5	Do.
227	3026	Jan. 28	Spleen 3016	Feb. 4	7	Do.
228	3039	Feb. 4	Spleen 3026	Feb. 7	3	Do.
229	3048	Feb. 7	Spleen 3039	Feb. 11	4	Do.
230	3058	Feb. 11	Spleen 3048	Feb. 18	7	Do.
231	3079	Feb. 18	Spleen 3058	Feb. 21	3	Do.
232	3086	Feb. 21	Spleen 3079	Feb. 25	4	Do.
233	3098	Feb. 25	Spleen 3086	Mar. 1	4	Do.
234	3099	Mar. 1	Spleen 3098	Mar. 4	3	Do.
235	3100	Mar. 4	Spleen 3099	Mar. 6	2	Do.
236	3101	Mar. 6	Spleen 3100	Mar. 11	5	Do.
237	3104	Mar. 11	Spleen 3101	Mar. 15	4	Do.
238	3107	Mar. 15	Spleen 3104	Mar. 18	3	Do.
239	3112	Mar. 18	Spleen 3107	Mar. 12	3	Do.
240	3115	Mar. 21	Spleen 3112	Mar. 25	4	Do.
241	3116	Mar. 25	Spleen 3115	Mar. 30	5	Do.
242	3118	Mar. 30	Spleen 3116	Apr. 3	4	Do.
243	3120	Apr. 3	Spleen 3118	Apr. 8	5	Do.
244	3121	Apr. 8	Spleen 3120	Apr. 13	5	Do.
245	3134	Apr. 13	Spleen 3121	Apr. 19	6	Do.
246	3137	Apr. 19	Spleen 3134	Apr. 24	5	Do.
247	3138	Apr. 24	Spleen 3137	Apr. 29	5	Do.

As mentioned above, Otto showed that it was not possible to increase the pathogenicity of strains of pest bacilli of moderate virulence by repeated passages through animals. The strains with which he performed his experiments were all capable of killing guinea pigs in moderate doses at the time of the commencement of the work.

I have attempted to increase the virulence of a more attenuated culture of the pest bacillus (one which in large doses was not capable of killing guinea pigs) by experiments performed on monkeys. The strain "Avirulent Manila" was employed for the inoculations. This organism was chosen because it had at an earlier time possessed a much greater virulence and because its pathogenicity had been reduced artificially. (See p. 310.) It was therefore interesting to see if the virulence could be reclaimed.

Experiments of this nature were first undertaken with the idea of ascertaining how long the attenuated organisms would remain alive in the tissues after subcutaneous inoculation. The technique of the experiments was as follows:

The abdomen of a monkey was first shaved and a suspension of the organism inoculated subcutaneously. The skin was then carefully massaged until the fluid was apparently completely absorbed. After varying periods of time, the skin of the abdomen was scrubbed several times with ether and alcohol and a small incision made with a sterile knife through the dermis. Cultures were then taken from the drops of blood or from the pus which escaped from the incised wound.

Usually, when the injection was made beneath the skin of the abdomen, a few hours later about the point of inoculation an œdematous swelling appeared which did not entirely disappear for forty-eight hours. In an earlier series of experiments made with a slightly more attenuated pest culture, it was found that the organisms were still very numerous in the tissues six to eight hours after the inoculation, after which time they gradually disappeared so that cultures made twenty-four hours subsequent to the injection generally, though not always, remained sterile. It seemed probable that the more resistant organisms were those which remained alive in the tissues for the longest time and that there was a true "survival of the fittest." An attempt was therefore made to ascertain if it would be possible to increase the pathogenicity of a slightly more virulent culture by this same procedure. As soon as the cultures made on agar from the incision in the animal developed, they were inoculated subcutaneously into a second monkey and so on throughout the series. The strain used for this series of experiments ("Avirulent Manila") was so attenuated at the time of their commencement that one 24-hour agar slant culture usually, but not invariably, caused death from subacute plague infection in a guinea pig of about 250 grams weight. This culture was passed through twenty-five monkeys according to the method above described. At the end of this time its virulence was found to be practically unchanged. The details of all the passages are given in Series 28 which follows. The monkeys employed were all of small size. The cultures were made from the animal, from one and one-half to twenty-four hours after the time of the inoculation. In a few instances in which the quantity of inoculated bacteria was large, pus formation about the point of injection was found to have occurred on incising the skin from twenty-one to twenty-four hours after the time of the infection of the animal. However, the organisms in the pus did not prove infectious when inoculated into guinea pigs in a considerable amount.

Therefore, my attempts to increase the virulence of a very virulent pest strain or to reclaim that of a more attenuated one have been entirely unsuccessful, the organisms in each instance having retained a very stable virulence throughout.

SERIES 28.—*Showing attempts to increase the virulence of the strain "Pest Avirulent Manila."*

Animal No.	Date and hour of inoculation.	Inoculated subcutaneously with—	Date and hour culture taken.	Result of culture.	Result to animal.	Microscopical specimens from wound.	Autopsy and remarks.
1581	Dec. 11 at 9 a. m.	One 24-hour agar slant culture suspended in 1 cc. bouillon.	Dec. 11 at 10.30 a. m. after 1½ hours.	Almost uniform growth of colonies over surface of slant.	Alive	A number of bacilli still present.	
1582	do	do	Dec. 11 at 1.30 p. m. after 4½ hours.	1 tube 114 colonies; in another 24 colonies.	do		
1589	Dec. 13 at 9 a. m.	One 48-hour agar culture obtained from monkey No. 1581 and one 48-hour agar culture obtained from monkey No. 1582, containing 114 colonies. These two cultures together suspended in 1 cc. of bouillon.	Dec. 13 at 2 p. m. after 5 hours.	5 cultures made. All developed innumerable colonies. The colonies appear as numerous as they usually do in colonies from the heart's blood of guinea pigs dead of pest infection.	Dead Feb. 5.	A few bacilli still present.	No bacilli in smears from spleen. 2 cultures from spleen negative. Animal much emaciated. No local lesion about point of inoculation apparent. Wound perfectly healed. Cause of death obscure.
1590	Dec. 15 at 10.20 a. m.	5 of the agar cultures (48 hour) which developed from monkey No. 1589 all together suspended in 1 cc. bouillon. The 5 cultures = about 1¼ full-grown agar slant cultures.	Dec. 15 at 4.30 p. m. after 6 hours.	Cultures show very numerous colonies of pest bacilli. Almost the whole surface of the slant covered.	Dead Jan. 17.*	Typical pest bacilli present.	Miliary and conglomerate tubercles in liver, spleen and lungs. Microscopical specimens show no plague bacilli. A few tubercle bacilli in smears from lungs. 2 cultures from the spleen and 2 from the heart, negative.*

*This is the only monkey found to be suffering with tuberculosis during the course of this research. Tuberculosis is very rare in monkeys in the Philippine Islands.

SERIES 28.—*Showing attempts to increase the virulence of the strain "Pest Avirulent Manila"*—Continued.

Animal No.	Date and hour of inoculation.	Inoculated subcutaneously with—	Date and hour culture taken.	Result of culture.	Result to animal.	Microscopical specimens from wound.	Autopsy and remarks.
1593	Dec. 18 at 9 a. m.	One 3-day agar slant culture from monkey No. 1590 suspended in 1 cc. bouillon.	Dec. 18 at 4.30 p. m. after 7½ hours.	Colonies nearly cover whole surface of slant.	Alive and well.	----------	Although the injection was made rather deeply into the abdominal muscles and all the fluid injected was quickly absorbed, nevertheless at 4.30 p. m., when the cultures were taken, an area of considerable œdema had developed over the abdomen about the region of the inoculation.
1608	Dec. 20 at 10.30 a. m.	1 agar slant 48-hour culture from monkey No. 1593.	Dec. 20 at 4.30 a. m. after 6 hours.	Very numerous colonies	Dead Jan. 9.	----------	In smears from the spleen it appeared that a few swollen bacilli were present. However, in two cultures from this organ in which a large amount of material was inoculated no growth took place.
1609	Dec. 20 at 10.30 a. m.	One 48-hour agar slant culture from monkey No. 1593 in 1 cc. bouillon.	Dec. 21 at 10.30 a. m. after 24 hours.	Culture negative	Dead Jan. 15	----------	No evidences of pest infection. 2 cultures from spleen and 2 from heart negative.
1611	Dec. 22 at 10 a. m.	Two 48-hour cultures from monkey No. 1609 in 1 cc. bouillon.	Dec. 22 at 4 p. m. after 4 hours.	Numerous colonies developed.	do	----------	Much emaciated. Nothing to suggest plague at autopsy. 2 cultures from spleen negative.
1626	Dec. 27 at 11 a. m.	One 5-day culture from monkey No. 1611 in 1 cc. bouillon.	Dec. 27 at 4.15 p. m. after 5 hours.	About 10 or 12 colonies on entire culture.	Alive	----------	

1629	Dec. 29 at 11 a. m.	One 48-hour culture from monkey No. 1626, equal to about $\frac{1}{10}$ of a full agar slant in 1 cc. bouillon.	Dec. 29 at 3 p. m. after 4 hours.	Fair number of colonies developed.	do	Bacilli still present	
1633	Jan. 3 at 10.30 a. m.	One 5-day culture from monkey No. 1629 in 1 cc. bouillon.	Jan. 3 at 3.30 p. m. after 5 hours.	Culture from drop of blood developed only a few colonies. Culture from deeper tissues developed very numerous colonies. Nearly whole surface of agar slant covered.	do		
1642	Jan. 5 at 11.30 a. m.	One 48-hour culture full grown slant from No. 1633 in 0.5 cc. bouillon.	Jan. 5 at 4.30 p. m. after 5 hours.	Culture from incision extending into muscle. Very rich growth of pest bacilli.	do		
1644	Jan. 6 at 10 a. m.	One 16-hour culture from monkey No. 1642 in 1 cc. bouillon.	Jan. 6 at 1 p. m. after 3 hours.	Numerous colonies	do		
1647	Jan. 8 at 10.30 a. m.	One 48-hour agar slant from monkey No. 1644 in 1 cc. bouillon.	Jan. 8 at 4 p. m. after 5½ hours.	do	do		Jan. 10, a guinea pig (No. 1652) was inoculated subcutaneously with ¾ agar slant (second generation from monkey No. 1647) suspended in 1 cc. bouillon. Animal remained well. Evidently no marked increase in virulence of the organisms.
1651	Jan. 10 at 10.30 a. m.	One 48-hour agar slant from monkey No. 1647 in 1 cc. bouillon.	Jan. 10 at 4 p. m. after 5½ hours.	Rich growth of pest bacilli. Very nearly whole surface of slant covered.	do		

SERIES 28.—*Showing attempts to increase the virulence of the strain "Pest Avirulent Manila"*—Continued.

Animal No.	Date and hour of inoculation.	Inoculated subcutaneously with—	Date and hour culture taken.	Result of culture.	Result to animal.	Microscopical specimens from wound.	Autopsy and remarks.
1654	Jan. 12 at 10 a. m.	One and one-half 48-hour agar slant from monkey No. 1651 in 1 cc. bouillon.	Jan. 12 at 4 p. m. after 6 hours.	Rich growth	Alive		
1670	Jan. 15 at 10.30 a. m.	One 3-day culture from monkey No. 1654 suspended in 1 cc. bouillon.	Jan. 15 at 4.30 p. m. after 6 hours.	do	Alive and well.	Cover slip	
1672	Jan. 16 at 4 p. m.	One and one-half 24-hour culture from monkey No. 1670 suspended in 1 cc. bouillon.	Jan. 17 at 9 a. m. after 17 hours.	do	do	Numerous organisms still present. Many enclosed in leucocytes.	
1675	Jan. 18 at 4 p. m.	One 30-hour agar slant culture from monkey No. 1672 suspended in 1 cc. bouillon.	Jan. 19 at 9.30 a. m. after 17½ hours	Cultures from drops of blood which oozed from the wound developed but very few colonies. Cultures from deeper tissues showed numerous colonies but these colonies became contaminated and could not be used for further inoculation.	Alive	Microscopical preparation from the blood which oozed from the incision showed no bacilli. On making a deeper incision and securing other specimens many bacteria were found. A large number were engulfed in leucocytes.	
1699	Jan. 22 at 11.45 a. m.	One 48-hour agar slant culture from monkey No. 1675, second generation, in 1 cc. bouillon.	Jan. 22 at 4 p. m. after 4 hours.	Rich growth	Alive and well.		

1701	Jan. 23 at 4 p. m.	One 24-hour agar culture from monkey No. 1699 in 1 cc. bouillon.	Jan. 24 at 10 a. m. after 18 hours.	do	do	Microscopical smears show numerous pest bacilli. Many engulfed in leucocytes.	
1706	Jan. 25 at 3 p. m.	One 24-hour agar culture from monkey No. 1701 in 1 cc. bouillon.	Jan. 26 at 9 a. m. after 18 hours.	Rich growth. Colonies almost entirely confluent.	do	Few organisms present.	
1710	Jan. 27 at 1 p. m.	One 24-hour agar culture from monkey No. 1706 in 1 cc. bouillon.	Jan. 28 at 10 a. m. after 21 hours.	Rich growth	do	----------	
1713	Jan. 29 at 4 p. m.	One 24-hour agar culture from monkey No. 1710 in 1 cc. bouillon.	Jan. 30 at 9. a. m. after 17 hours.	do	do	A few bacilli seen. All extracellular.	
1715	Jan. 31 at 4.30 p. m.	One 24-hour agar culture from monkey No. 1713 in 1 cc. bouillon.	Feb. 1 at 9 a. m. after 16½ hours.	do	do	Very numerous pest bacilli in smear from blood from incision.	
1723	Feb. 2 at 3 p. m.	One 24-hour agar culture from monkey No. 1715 in 1 cc. bouillon.	Feb. 3 at 9 a. m. after 18 hours.	do	do	Numerous pest bacilli in smears from incision. Many within leucocytes.	
1726	Feb. 4 at 9.30 a. m.	One and one-half 24-hour cultures from monkey No. 1723 in 1 cc. bouillon.	Feb. 5 at 9 a. m. after 23 hours.	do	do	Microscopical preparation showed many pus cells containing numerous pest bacilli. Also free organisms.	On Feb. 5, on incision of the skin over the point of inoculation, a small pocket of grayish (not yellow) pus was encountered. The abdomen of guinea pig No. 1727 was shaved and scarified with a scalpel and several loops of this pus and blood rubbed over the scarified area. The animal remained well.

SERIES 28.—*Showing attempts to increase the virulence of the strain "Pest Avirulent Manila"*—Continued.

Animal No.	Date and hour of inoculation.	Inoculated subcutaneously with—	Date and hour culture taken.	Result of culture.	Result to animal.	Microscopical specimens from wound.	Autopsy and remarks.
1730	Feb. 7 at 10.30 a. m.	Two 48-hour agar slant cultures from monkey No. 1726 in 1 cc. bouillon.	Feb. 8 at 8.45 a. m. after 10 hours.	Rich growth	Alive and well.	Very numerous pest bacilli in smear of blood from incision; both intra-and extra-cellular; numerous pus cells.	
1746	Feb. 10 at 12.30 a. m.	One 48-hour full agar slant culture from monkey No. 1730 in 1 cc. bouillon.	Feb. 11 at 9.30 a. m. after 21 hours.	do	do	Numerous pest bacilli in pus which oozed from incision. Many within leucocytes.	On Feb. 11, on incision over the point of inoculation where a distinct swelling had occurred, considerable pus escaped.
1757	Feb. 12 at 9.30 a. m.	One and one-half 24-hour agar slant cultures from monkey No. 1746 in 1 cc. bouillon.	Feb. 13 at 9.30 a. m. after 24 hours.	do	do	Numerous pest bacilli in drops of blood from incision.	A considerable swelling developed about the point of inoculation and on Feb. 13, on incising this area to make cultures, blood, pus, and serum escaped. About 0.2 cc. of this material was drawn up in a syringe and inoculated beneath the skin of the abdomen of guinea pig No. 1758. The animal remained entirely well.
*1760	Feb. 14	One 30-hour full agar slant culture from monkey No. 1757 suspended in 1 cc. bouillon, inoculated subcutaneously.			do		Evidently there has been no increase of virulence.

*Guinea pig, 240 grams' weight.

ARTIFICIAL ATTENUATION OF THE PLAGUE BACILLUS.

In spite of the facts which have been brought out in the previous discussion, nevertheless it is true that the virulence of different pest strains as they occur in nature varies greatly, the numerous cultures isolated from different epidemics and different cases of human plague frequently exhibiting marked variation in pathogenicity in animal experiments. It is also true that there are a number of references in the literature which point to the fact that certain strains of pest bacilli, after growing for a few generations on artificial culture media, unaccountably lose their virulence. It therefore is not my purpose to argue that under certain conditions the virulence of the pest bacillus may not vary from time to time and that it may not frequently, under some conditions in nature, become attenuated and under others, such as its passage from animal to animal, even be increased. However, any such change as the latter is very unusual and it does not occur under ordinary circumstances. The important point which I wish to emphasize in the behavior of this organism in its passage from animal to animal is its stability of virulence, and this quality is also possessed to a remarkable degree by the organism on artificial culture media.

Virulent strains of the pest bacillus when grown upon artificial media usually suffer no change in their pathogenicity even after long periods of time and after numerous generations. Cultures sealed in test tubes and protected from light and heat have been found to retain their full virulence for several years. Thus Maassen found no change of virulence in cultures after two years and N. K. Schultze[110] observed that cultures in bouillon were fully virulent even after four years. Attempts to attenuate the virulent organism in cultures have also demonstrated its stability in pathogenicity and have usually resulted unsuccessfully.

Albrecht and Gohn[111] reported that the pest bacillus could resist a continued temperature of 36° C. for only about fourteen days without loss of virulence, and that after this period a rapid diminution occurred. However, these results have not been confirmed by any other observers. The German Plague Commission[112] suspended agar cultures of the organism in bouillon and exposed them to a temperature of 51° C. for varying periods of time. They found that while many of the organisms were killed by this treatment, those which remained alive had retained their full virulence. They also attempted to secure attenuation of the organism by exposing it to carbolic acid, but these experiments were also unsuccessful.

Kolle and Otto[113] found that by growing the organism at a continuous temperature of 40° to 41° C. a further attenuation was sometimes possible in cultures which already were of a low grade of virulence.[114] Hetsch has experimented extensively with the question of the artificial attenuation of the plague organism. He found that prolonged growth of the bacilli at temperatures ranging from 40° to 45° C. caused no attenuation of the surviving organisms. Cultivation of the germ in an insusceptible animal such as the frog (in its dorsal lymph sac) also caused no lowering of the virulence. After experimenting with a number of chemical substances, particularly with various staining reagents, he found that the most satisfactory means of attenuation consisted of cultivating the organism in flasks of bouillon to which increasing amounts of alcohol were gradually added.

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[110] *Centrbl. f. Bakteriol.* (1901), **29**, Abt. I, 169.

[111] *Loc. cit.*

[112] *Loc. cit.*

[113] *Deutsche med. Wchnschr.* (1903), **29**, 493.

I have been successful in attenuating two strains of pest bacilli by employing the method recommended by Hetsch. My cultures in the alcoholic bouillon, in addition, were frequently grown at a temperature of from 41° to 43° C. The strains used for the experiments were those designated as "Pest Virulent" and "Pest Avirulent Manila." The organism was first inoculated into a 50 cubic centimeter flask containing bouillon; after forty-eight hours' growth at 30° C. the alcohol was added and the flask placed at a temperature of 41° to 43° C. for a period usually of three weeks. Cultures from the flask were then inoculated upon agar for many generations, a fresh one being made each day, sometimes for one or two weeks, and the organism usually cultivated at the same high temperature. Beginning with 0.1 cubic centimeter of absolute alcohol and 50 cubic centimeters of bouillon, the amount of the former was gradually increased in successive cultures up to 5 cubic centimeters in 50 cubic centimeters of bouillon. In some instances the alcohol in the flask was increased each week and in others every few days. The organisms in the flask containing the larger amounts of alcohol frequently perished entirely and in other instances, when inoculations were made from them upon agar only, a few isolated colonies developed in the medium. If the former took place, the cultures were obviously lost and the last step of the attenuation had then to be repeated with the previous transplant of the more virulent organism.

This method of attenuation does not give quick results, and as Hetsch admits, it is frequently not satisfactory for diminishing the virulence of the plague organism. Moreover, it sometimes appears to be very inefficient in bringing about this change when the strains are of very great virulence. However, it would appear to be the most successful method which has yet been discovered. The time during which the experiments were carried on in the attenuation of the strain "Pest Avirulent Manila" was a little over seven months, and in the strain "Pest Virulent" about seventeen months.

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[114] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1904), **48**, 443.

At the time of the beginning of the attenuation the strain "Pest Avirulent Manila," when inoculated subcutaneously, killed guinea pigs of from 200 to 300 grams weight in doses of about 2 oesen. At the time the experiments were discontinued, the lethal dose for guinea pigs of the same weight was about one agar slant culture, although the animal sometimes even survived such a dose.

The organism during the time of its attenuation was grown for five different periods in the bouillon containing the different percentages of alcohol. Series 59 (p. 312) gives the details of the change in virulence.

At the time of the beginning of the experiments in the attenuation of the strain "Pest Virulent" the organism possessed a virulence so great that a portion of a spleen of a guinea pig which had died from infection with it, when it was rubbed over the abdomen of a second guinea pig, always caused the death of the animal from acute plague infection, and 5 oesen of a suspension (prepared by adding 5 cubic centimeters of saline solution to a 24-hour agar slant culture of the organism) when rubbed over a shaved and scarified area on the abdomen of a guinea pig, also invariably caused it to succumb to plague. After the virulent strain had been grown for nearly three months in the alcoholic bouillon, ½ oese of a 24-hour agar culture of the organism when injected subcutaneously into a guinea pig, still caused its death; after the experiment had been carried on for eleven months the virulence of the organism was found to be such that 2 oesen of the 48-hour agar culture, when rubbed over a shaved and scarified area on the abdomen of a guinea pig, or the same amount injected subcutaneously, no longer caused the death of the inoculated animals. However, ½ agar slant after two months more of attenuation, invariably caused fatal infection. At the time of the termination of the experiments which lasted for about seventeen months, the virulence was so reduced that ½ agar slant culture of the organism, when injected subcutaneously into guinea pigs, did not cause the death of more than 40 per cent of the animals so inoculated, the remaining 60 per cent remaining alive and well. At the termination of the experiment the same original strain (unattenuated) which had been kept in a sealed tube and protected from light during the same period of time, was grown for two successive transplants upon fresh agar and its virulence again tested. It was found that 0.1 oese of this culture, when rubbed over the scarified abdomen of a guinea pig, still caused the death of the animal, showing that no practical decrease in the virulence of the organism had as yet occurred. The details of the attenuation of the strain are recorded in the series of experiments numbered 60.

SERIES 59.—*Showing the attenuation of the strain "Pest Avirulent Manila," in guinea pigs.*

Animal No.	Inoculated.	Result.	Autopsy.
1164	June 28, two oesen strain "Pest Avirulent Manila" subcutaneously.	Dead July 3, after 5 days.	Numerous pest bacilli in smears from spleen.
1166	July 10, two oesen strain "Pest Avirulent Manila" subcutaneously.	Dead July 16, after 6 days.	Do.
1450	Nov. 14, one-half agar slant culture "Pest Avirulent Manila" (attenuated 5 months) subcutaneously.	Alive and well.	
1578	Dec. 9, one 48-hour agar slant culture "Pest Avirulent Manila" (attenuated 6 months) subcutaneously.	Dead Dec. 18, after 9 days.	Few involution forms of plague bacilli in the heart's blood. Six colonies developed in one culture and none in the second.
1655	Jan. 12, three-fourths 24-hour agar slant culture "Pest Avirulent Manila" (attenuated 7 months) subcutaneously.	Alive and well.	
1671	Jan. 15, one 48-hour agar slant culture "Pest Avirulent Manila" (attenuated 7 months) subcutaneously.	Dead Jan. 29, after 14 days.	Subacute pest.
1751	Feb. 11, one 30-hour agar slant "Pest Avirulent Manila" (attenuated 7½ months) subcutaneously.	Alive and well.	

SERIES 60.—*Showing the attenuation of the strain "Pest Virulent," in guinea pigs.*

Animal No.	Inoculated.	Result.	Autopsy and remarks.
1497	Nov. 27, abdomen shaved and rubbed with spleen of guinea pig No. 1495, just dead of plague from inoculation of the strain "Pest Virulent."	Dead Dec. 2, after 5 days.	Numerous pest bacilli in smears from spleen.
1763	Feb. 16, one-half oese "Pest Virulent" (attenuated 3 months) subcutaneously.	Dead Feb. 20, after 4 days.	Pest.
2068	June 7, one oese "Pest Virulent" (attenuated 6 months) subcutaneously.	Dead June 12, after 5 days.	Do.
2069	-----do-----	Dead June 14, after 7 days.	Do.
2070	-----do-----	Dead June 12, after 5 days.	Do.
2071	-----do-----	Dead June 11, after 4 days.	Do.
2072	-----do-----	Dead June 14, after 7 days.	Do.
2194	July 23, one oese "Pest Virulent" (attenuated 8 months) subcutaneously.	Alive and well	
2195	-----do-----	-----do-----	
2196	-----do-----	Dead Aug. 4, after 12 days.	Subacute pest.
2592	Oct. 25, two oesen "Pest Virulent" (attenuated 11 months) subcutaneously.	Alive and well	
2593	-----do-----	-----do-----	

SERIES 60.—*Showing the attenuation of the strain "Pest Virulent," etc.*—Continued.

Animal No.	Inoculated.	Result.	Autopsy and remarks.
3029	Jan. 29, one agar slant culture "Pest Virulent" (attenuated 14 months) subcutaneously.	Dead Feb. 5, after 7 days.	Large suppurating bubo in abdominal wall.
3030	-----do-----	Dead Feb. 4, after 6 days.	Large cyst-like bubo.
3031	Jan. 29, one-half agar slant culture "Pest Virulent" (attenuated 14 months) subcutaneously.	Dead Feb. 11, after 13 days.	Advanced pest pneumonia.
3032	-----do-----	Dead Feb. 9, after 11 days.	Subacute pest.
3033	Jan. 29, one-fourth agar slant culture "Pest Virulent" (attenuated 14 months) subcutaneously.	Alive and well--	
3034	-----do-----	-----do-----	
3139	Apr. 25, one-half agar slant culture "Pest Virulent" (attenuated 17 months) subcutaneously.	Dead May 6, after 11 days.	Large bubo. Subacute pest.
3140	-----do-----	Alive and well--	
3141	-----do-----	-----do-----	
3142	-----do-----	-----do-----	
3143	-----do-----	-----do-----	
3144	-----do-----	-----do-----	
3145	-----do-----	Dead May 6, after 11 days.	Subacute pest.
3146	-----do-----	Dead May 3, after 8 days.	Do.
3147	-----do-----	Dead Apr. 26, after 1 day.	Not of pest infection.
3148	-----do-----	Alive and well--	
3149	Apr. 25, one-tenth oese "Pest Virulent" (original) rubbed over abdomen.	Dead Apr. 30, after 5 days.	Acute pest infection.

STABILITY OF VIRULENCE OF THE PLAGUE BACILLUS.

These experiments, relating to the artificial attenuation of the pest bacillus in cultures, as well as those performed on animals with the view of increasing the pathogenicity of this organism all demonstrate its great stability of virulence.

In respect to this phenomenon, it is interesting to compare the behavior of the cholera organism with that of the pest bacillus, both in its growth on artificial media and in successive passages through the animal body. The cholera organism if left for a few months on culture media such as agar, soon loses its virulence and indeed, frequently dies out, largely owing to the autolytic digestion of the bacteria which takes place. If, instead of allowing the spirillum to remain in the original tube in which it was inoculated, it is transplanted for a number of times on agar, a rapid loss of virulence is also obtained. On the other hand, this virulence can quickly again be increased by successive passages of the culture through a series of a species of animal such as the guinea pig, and in from fifteen to twenty-five successive passages the maximum virulence of the spirillum can usually be reached if the attenuation has not been too great. Again, if such a culture which has attained its maximum virulence be left upon artificial media for a few weeks, the virulence becomes greatly reduced. This process of increasing and of decreasing the virulence of the cholera organism may be repeated at will.

On the other hand, we have seen with the pest bacillus that it is impossible to reproduce these phenomena. The pest bacillus does not easily undergo autolysis in old agar cultures and it produces no ferments which are capable of causing its own destruction. Virulent pest cultures, whether they remain continuously in the original agar culture upon which they were first inoculated or are transplanted from culture to culture, remain alive and frequently retain their full virulence, although many instances of spontaneous loss of this property occur. Strains of the organism of moderate virulence, but which still kill guinea pigs, can not be rendered perceptibly more virulent by repeated passages from animal to animal, unless perhaps it be under certain special conditions and the same applies to avirulent strains of the organism which rarely kill the animal except in very large doses.

This evidence is particularly interesting from an epidemiological standpoint, when we compare epidemics of cholera with those of plague. With the former disease, the epidemic—no matter which type it may later assume—probably begins either by the introduction of the virulent organism into a new district by a sick individual who has traveled from a region where the disease is already epidemic, or by a few cases of infection in the neighborhood of the new district, which are usually of a mild character and may entirely fail to attract attention. In case the water supply of the region becomes infected from some of these cases, the organism having increased in virulence in its passage through the more susceptible individuals with which it has come in contact, the epidemic may assume an explosive character which is very marked and thousands of people may be stricken within a day or two. If the epidemic does not extend through the water supply, but by direct or indirect contact with cases of infection, a better opportunity for its study is usually afforded. It may then frequently be observed, although obviously this is not always the case, that as the epidemic extends the virulence of the cases increases to a maximum and then as the number of cases slowly subsides, the severity also usually becomes reduced, so that within a short period of time, at most within a year or two, the epidemic has either reached its maximum severity or has entirely disappeared. Such outbreaks of cholera would appear partially to be due (leaving meteorological influences, relation to soil, dissemination of the organism, etc., out of the question), to a rise of virulence of the cholera spirillum which is brought about by the fact that the more or less attenuated organism has come in contact with a number of particularly susceptible individuals and the period of the epidemic, under certain conditions at least, seems to be limited particularly by the virulence of the organism and by the number of susceptible individuals which continue to be infected with it. As the virulence of the organism diminishes, the number of cases of cholera in a community will diminish. Avirulent cholera organisms, when grown on culture media in the laboratory, are practically harmless when ingested by all but very susceptible human beings and the same is probably true of the more attenuated spirilla which occur in nature. It would then appear that the instability of the virulence of the cholera organism must frequently play an important rôle in determining the character and duration of the cholera epidemic.

How different is the picture in at least some plague epidemics, such, for example, as that which has been witnessed in India for the past ten years and in which the number of deaths reported each year has almost steadily increased from 1897 to 1904–5. The reported deaths in India from this disease, according to the Indian Medical Gazette of July, 1906, are as follows:

	Deaths.
Up to end of 1897	57,965
1898	118,103
1899	134,102
1900	91,627
1901	273,679
1902	577,427
1903	851,263
1904	1,022,300
1905	950,863
1906 to end of April	170,000

It can not be argued that the disease is becoming any more or any less virulent from year to year, judging from the death rate, for we know that it is still spreading and that it has not even yet extended over the whole of India. Burma has escaped until within the last few months, but is now suffering very severely.

Knowing as we do from laboratory experiments that the plague bacillus is frequently extremely stable in its virulence in nature and that it may not easily become attenuated under artificial conditions, knowing also that the epidemic of this disease in India has not decreased in a period of ten years, the outlook for the extermination of the malady in that country by any other means than by the exhaustion of suitable susceptible individuals and animals does not now seem to be hopeful.

It is for this reason that I agree with Professor Haffkine, Colonel Bannermann and many others in the idea that it is certain that protective inoculation against plague must become one of the most effective means that can be employed in India for the extermination of the malady. And while in combating this disease the ordinary hygienic measures should in my opinion certainly not be neglected and special attention should be given to disinfection and particularly to the destruction of rats and fleas, yet it must be borne in mind that in spite of all these precautions plague may continue to exist as it has and one might almost say will continue to exist in India until the larger portion of the susceptible population is either protected artificially by vaccination or naturally by an attack of the disease, or until the agents which transmit the malady become exhausted.

As the disease spreads and the organism becomes widely disseminated in new districts and new countries, if the same conditions for the transmission of the malady are to be encountered in the newly infected regions, we must not be surprised if the same virulence and the same high mortality continue. Only through the destruction of the bacillus and of the means by which it is conveyed to man (rats, fleas, etc.) and through the exhaustion of the supply of the more susceptible human beings, does it appear that some epidemics of plague may be limited. As long as the organisms in these epidemics are sufficiently disseminated and properly conveyed to man, the epidemics may continue for indefinite periods. In other words, there is not the same hope of a plague epidemic wearing itself out, so to speak, as there is of a cholera epidemic doing so.

On the other hand, it is well known that the severe epidemics of the disease may be preceded by sporadic cases of mild plague, and that glandular swellings in cases free of fever have also frequently been reported to exist, before a more general outbreak of plague appears in malignant form. The malady may also increase in virulence if it occurs in the same locality in successive years, as was witnessed in the epidemic at Bombay in 1896–97. We also know, as has been pointed out by Simpson,[115] that plague may begin in a new locality, then pass into a very virulent variety and after reaching epidemic proportions, gradually return to a mild form of the disease; or, on the other hand, that some epidemics may be mild and others malignant throughout.

In this connection it is interesting to observe that J. F. Payne[116] goes so far as to distinguish two distinct strains of plague: First, the Eastern Asiatic strain, distinguished by—

> (1) The frequent occurrence of epidemics of *Pestis minor* or mild plague; (2) the absence of any observed connection with the epizoötic disease of rodents; (3) more self-limited epidemics; (4) consequently less marked power of extension, for the eastern Asiatic epidemics have not in modern times traveled very far; (5) on the whole less virulence and a lower percentage mortality.

[115] Treatise on Plague (1905), 159. Cambridge.

[116] Allbutt & Rolleston, System of Medicine (1907), 2, 388.

Second, the Indo-China strain which, in contrast to the first, is distinguished by—

(1) An almost invariable connection with great mortality among rats and less frequently among other animals; (2) less frequent occurrence of epidemics of *Pestis minor,* although it is possible that further observations may show that these are commoner than has been supposed; (3) remarkable power of extension as shown in its spread over a large part of India and conveyance to many other countries; (4) in general, more intense virulence and higher case mortality; (5) the pneumonic form accompanied by hæmoptysis is much commoner.

However, the variations in virulence of the disease are dependent somewhat upon the differences in the susceptibility of the individual attacked and are not entirely connected with the degree of virulence of the organism, which as we have seen from a discussion of this subject, may change under some of the physical conditions it meets in nature and in certain of the passages through the animal body.

Therefore, it must unquestionably be admitted that the pest bacillus may under certain conditions become attenuated many times during the course of an epidemic, and under certain others regain its virulence. Nevertheless, the general stability of its virulence must be recognized, and I believe that it is this quality which may markedly influence the nature and course of at least some epidemics of plague.

The plague organism in nature is not very resistant and easily becomes destroyed under certain conditions, but it frequently does not easily become attenuated, either in the animal body or outside of it.

RELATION BETWEEN THE VIRULENCE OF THE PLAGUE BACILLUS AND ITS IMMUNIZING POWER.

The question of the relation between the virulence and the immunizing power of different strains of the plague bacillus obviously is an important one in its bearing upon the subject of protective inoculation, particularly with reference to that form in which the living, attenuated organism is employed. The subject of virulence in relation to the immunizing power of microörganisms in general has attracted considerable attention during the past few years. Pfeiffer, Friedberger[117] and myself[118] found that with cholera spirilla a greater immunity was obtained with the more virulent organism. Pfeiffer and Friedberger employed four strains in their investigations. My experiments were carried on with two strains of cholera spirilla of widely different virulence, and I was able conclusively to show that the virulent organism upon inoculation produced a higher immunity and at the same time bound a greater number of amboceptors in a cholera immune serum than did the avirulent one. At the time of the publication of these experiments I stated that "these conclusions apply to the two strains of cholera spirilla employed in the foregoing experiments. Whether they will also hold good with other strains of this spirillum or for microorganisms in general must be decided by further experimental work."

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[117] *Berl. Klin. Wchnsch.* (1902), **39**, 581.

[118] *Publications of the Bureau of Government Laboratories,* Manila (1904), No. 21; 1 *J. Exp. Med.* (1905), **7**, 229.

Wassermann,[119] in studying the question with typhoid bacilli found that two strains of the organism which possessed a greater immunizing power and a greater power of binding amboceptors than a third strain, were nevertheless less virulent. From these experiments one would conclude that the immunizing power depended rather upon the binding power of the organism than upon its virulence. During the past year Meinicke, Jaffé and Flemming,[120] have performed careful and much more extensive experiments with cholera spirilla, their investigations relating to the question of virulence with reference to the power to bind amboceptors and to immunize. While in some instances they found that a virulent cholera culture was able to bind more amboceptors than an avirulent one, in the great majority of their experiments they were able to show that the binding power was independent of virulence, since in many instances the less virulent culture bound a greater number of amboceptors than the more virulent one.

Their experiments referring to the question of the relation between virulence and immunizing power are not numerous, as the authors admit. This seems to me to be unfortunate, for by means of their methods of examination and the large number of strains which they studied they were in a position to solve this problem conclusively. In one experiment they were able to show that, in one instance at least, a much attenuated culture was in a rabbit able to give rise to a serum of higher value than resulted from the use of a very virulent culture. Nevertheless, it would appear that an individual variation in immunity in the rabbit immunized with either the virulent or the avirulent organism, might also account for the difference in immunity obtained, and therefore it would appear to be injudicious to draw decided conclusions from this one result. Moreover, it may be seen in the table which Meinicke, Jaffé and Flemming compiled, and the fact is also emphasized in the text of their article, that with the small doses inoculated, 0.1 and 0.01 oese, there was very great variation in the value of the sera produced in the different rabbits. Thus, of two animals, each inoculated with 0.01 oese of the same culture, one furnished a serum showing a bactericidal value of 1:400 and the other of 1:1,000. However, it must be admitted that the experiments of these authors not only show that with the cholera organism binding power may be independent of virulence but they also suggest very strongly, if they do not prove conclusively, the fact that the immunizing power also may be independent of virulence.

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[119] Festschrift Robert Koch (1903), 503.

[120] *Ztschr. f. Hyg. u. Infectionskrank.*, Leipz. (1906), **52**, **416**.

Pfeiffer[121] found that in immunization against plague, the degree of immunity produced depended not only upon the dose of the killed pest culture but also upon the degree of virulence of the killed organism, and concluded that the immunizing effect of pest bacilli is, up to a certain extent, proportional to the virulence of the culture employed. Kolle and Martini[122] found that the less virulent strains of pest bacilli were agglutinated by an immune serum in higher dilutions than more virulent ones. However, Shibayama[123] could not confirm this fact.

In my experiments in agglutination of pest bacilli, the strain "Pest Avirulent" was agglutinated more easily than "Pest Virulent," but as pseudo-agglutinations occurred so frequently with the former strain, I am not willing to draw any conclusions from my experiments in regard to this question.

The pest bacillus is not a very favorable organism with which to study the relation between virulence and immunizing power, and a particularly unfavorable one with which to undertake binding experiments of the amboceptors in an immune serum. For this reason, I shall only briefly discuss the results which were obtained in immunization with several of the strains of different virulence. As may be seen from a number of the tables of inoculation, monkeys inoculated by thrusting subcutaneously a syringe needle which had been dipped in a suspension of the strain "Pest Virulent," frequently survived the inoculation and later showed themselves to be immune to pest infection. On the other hand, monkeys inoculated in the same manner, with a suspension of the strain "Pest Avirulent" always survived the inoculation, but later invariably showed no demonstrable immunity, and all succumbed to pest when reinoculated with a lethal dose of the virulent strain.

Throughout the numerous experiments performed in the vaccination of guinea pigs and monkeys with the strains "Pest Avirulent" and pest "Maassen Alt," it became clearly demonstrated that the latter organism was distinctly more virulent than the former. This fact may be confirmed from a study of the series of inoculated animals given in the tables, and also from them it may clearly be seen that the strain pest "Maassen Alt" produced a higher immunity in the animals which were inoculated with it than was brought about in those inoculated with the strain "Pest Avirulent." The following table summarizes these results:

Strain of plague bacillus.	Guinea pigs.			Monkeys.		
	Number inoculated with 1 agar slant culture.	Number died from inoculation.	Percentage found immune on subsequent test.	Number inoculated.	Number died from inoculation.	Percentage found immune on subsequent test.
"Pest Avirulent"	60	2	68.6	44	1	52
Pest "Maassen Alt"	47	6	88	49	5	70

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[121] *Berl. Klin. Wchnsch.* (1902), **39**, 581.

[122] *Deutsche med. Wchnsch.* (1902), **28**, 45.

[123] *Centrbl. f. Bakteriol. Orig.* (1905), **38**, 482.

Therefore, my experiments would appear to confirm the earlier suggestions of R. Pfeiffer that the immunizing power of the plague organism is, within certain limits, proportional to its virulence. However, as my work relating to this question was performed with only three strains of the pest bacillus, it can not be considered to be conclusive for this organism in general and it can merely be stated that with the strains which I have studied, the more virulent organism gave rise to the greater immunity, and that the immunity within certain limits was proportional to the dose. (See experiments, pp. 199 to 221.)

XIV. RELATION OF THE IMMUNITY REACTIONS BETWEEN PEST, RINDERPEST, AND HÆMORRHAGIC SEPTICÆMIA.

The relationship which certain of the immunity reactions encountered in the study of pest and rinderpest bear to one another is sufficiently close to attract attention. Perhaps the greatest resemblance is seen in the action of the immune serum in the two diseases, which in each instance is anti-infectious in nature, possessing but little curative power. In rinderpest as in plague, there is little hope of saving the life of the animal by use of the serum, if the infection is well advanced before the serum is inoculated. The highest immunity in each of these diseases is also obtained by the inoculation of the living organism.

However, the plague bacillus presents a more uniform structure in the arrangement of its receptors than many of the other organisms of the hæmorrhagic septicæmia group. Thus, for example, it has been found in swine plague that a serum which may show high protective power for mice against several strains of bacilli of this group, against others will in these animals exert almost no beneficial action whatsoever. This is not true in the case of pest. A plague immune serum produced with one pest strain possesses polyvalent properties in that it will exert its anti-infectious action against all strains of the pest bacillus no matter what their source, and a plague polyvalent serum is not more or less effective in its action against any one of these different strains of pest than is a univalent one.

Kolle, Otto and Hetsch[124] found that guinea pigs or rats, only in very exceptional instances, could be immunized against pest infection by treatment with large doses of other living bacilli of the hæmorrhagic septicæmia group, and, vice versa, that animals immune to pest infection rarely showed any immunity against these pest-like bacteria.

From these experiments it seems established that the receptors of the two groups of bacteria are specific and differ considerably from one another and that the immunity is also specific in each instance and does not depend upon the more or less non-specific stimulation of the leucocytes, as Terni [125] has suggested in his most recent publication on this subject.

In connection with the experiments relating to the immunity reactions between pest bacilli and the organisms of the hæmorrhagic septicæmia group, it may be mentioned that we have noticed in Manila that cattle which had been rendered thoroughly immune to rinderpest were not immune to hæmorrhagic septicæmia and frequently succumbed from the latter disease. The immunity here is also in each instance specific. From all these reasons and particuarly because apparently only ruminants are susceptible to rinderpest infection, it did not seem at all likely that guinea pigs which had been inoculated with virulent rinderpest blood or with antirinderpest serum would later show any immunity to pest infection, but since in testing antirinderpest serum in the laboratory the opportunity presented itself of carrying on these experiments, they will here be recorded. Ten guinea pigs (see Series 66) were inoculated intraperitoneally each with 0.2 cubic centimeter of virulent rinderpest blood, 0.1 cubic centimeter of which, inoculated on the same day, gave rise to a fatal rinderpest infection in a steer. The guinea pigs showed no unfavorable symptoms following the inoculation. From one to three weeks later the immunity of the animals to plague infection was tested by rubbing a portion of a plague spleen from a guinea pig just dead of pest over a shaved area of the abdomen of the animals to be tested. None of the guinea pigs of the series showed any plague immunity whatever, as may be seen from the table, and all succumbed to acute plague infection. Ten other guinea pigs (Series 67) were each inoculated subcutaneously with 2 cubic centimeters of fresh antirinderpest serum. Six days later the immunity against plague of the animals was tested by the same method and all died of acute plague infection from the second to the fourth day after inoculation. Therefore, no relationship in immunity between these two infections could be stablished by these experiments, and they gave no encouragement for the performance of similar experiments on the larger and more expensive animals, such as cattle.

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[124] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1904), **48**, 364.

[125] *Ibid.* (1906), **54**, 385.

SERIES 66.—*Guinea pigs inoculated with virulent rinderpest blood, followed by plague infection.*

Animal No.	Inoculated January 19 with virulent rinderpest blood intraperitoneally.	Infected by shaving abdomen and rubbing with—	Result.	Remarks.
1708	0.2. cc.	Pest spleen of guinea pig No. 1702, Jan. 26.	Dead Jan. 29, after 3 days.	Culture from heart, pest bacilli.
1709	do	Pest spleen of guinea pig No. 1703, Jan. 27.	Dead Feb. 6, after 10 days.	Do.
1711	do	Pest spleen of guinea pig No. 1704, Jan. 28.	Dead Feb. 4, after 7 days.	Do.
1712	do	Pest spleen of guinea pig No. 1708, Jan. 29.	Dead Jan. 31, after 3 days.	Do.
1714	do	Pest spleen of guinea pig No. 1712, Jan. 31.	Dead Feb. 4, after 4 days.	Do.
1724	do	Pest spleen of guinea pig No. 1711, Feb. 4.	Dead Feb. 8, after 4 days.	Do.
1725	do	Pest spleen of guinea pig No 1714, Feb. 4.	Dead Feb. 7, after 3 days.	Do.
1728	do	Pest spleen of guinea pig No. 1709, Feb. 7.	Dead Feb. 10, after 3 days.	Do.
1729	do	Pest spleen of guinea pig No. 1725, Feb. 7.	Dead Feb. 12, after 5 days.	Do.
1745	do	Pest spleen of guinea pig No. 1724, Feb. 8.	Dead Feb. 12, after 4 days.	Do.

SERIES 67.

Each guinea pig of this series was inoculated subcutaneously with 2 cubic centimeters of fresh antirinderpest serum. Six days later each animal was reinoculated by massaging a shaved area over the abdomen with a portion of a spleen from a guinea pig (No. 3058) just dead of plague infection.

Guinea pig No.	Antirinderpestic serum subcutaneously.	Infected 6 days after injection of serum.	Result.	Remarks.
3074	2 cubic centimeters.	With pest	Dead after 2 days	Numerous pest bacilli in smears from spleen.
3075	do	do	Dead after 4 days	Do.
3076	do	do	Dead after 2 days	Do.
3077	do	do	do	Do.
3078	do	do	do	Do.
3079	do	do	Dead after 3 days	Do.
3080	do	do	do	Do.
3081	do	do	Dead after 4 days	Do.
3082	do	do	Dead after 2 days	Do.
3083	do	do	do	

XV. PLAGUE VACCINATION IN HUMAN BEINGS.

It having been conclusively demonstrated by animal experiments that by the method of vaccination against plague a much greater immunity could be produced than by any other method of inoculation, and earlier experiments [126] having demonstrated the entire safety of the inoculation of human beings with large amounts of the strain "Pest Avirulent," more extensive vaccinations were made in man with this culture. The size of the dose employed for an adult was always one 24-hour agar slant culture and for children from one-third to one-half this quantity. Surprising as it may seem, the injection of these large amounts of the living plague organism have not given rise to any very severe reactions. A few hours after the inoculation, the temperature of the individual usually begins to rise. When the injections have been made in the morning, the fever may in the evening of the first day reach from 38.5° to 39° C. Only in a few cases has it touched 40°. The temperature gradually declines on the following day and by the third of fourth one, has become normal. Occasionally, the cases showed a moderate leucocytosis after the injection. As in the earlier experiments, the organisms were always suspended in 1 cubic centimeter of 0.085 saline solution and the inoculations were made deeply into the deltoid muscle. Intramuscular instead of subcutaneous injections were performed on account of the quicker absorption which occurs from the former method, and because Meltzer and Auer [127] found that intramuscular injections as regards absorption in general stand in value very near that of a direct injection into the circulation. On the day after the vaccination there is usually distinct induration and redness about the point of the injection with some soreness on pressure, but these symptoms subside in from one to three days. No visible suppuration of the tissues has ever occurred.

In order to observe the length of time during which the organisms remained alive in the tissues, as mentioned, similar inoculations were made in monkeys and the tissues near the point of injection incised at varying intervals after the vaccination, and microscopical preparations and cultures made from the drops of blood which escaped from the wound. The technique employed was the same as that used in the experiments relating to increasing the virulence of the plague organism, as described on page 301. In the different series of cultures made from the animals inoculated with the strain "Pest Avirulent" it was found that from six to eight hours after the time of the inoculation the organisms were still very numerous in the tissues, after which time they gradually diminished, and, usually after twenty-four hours, they were no longer reclaimable in cultures. However, in several instances a few colonies developed in the cultures made after this period of time. In immunized animals the organism was destroyed in a shorter period, the cultures made after twelve hours frequently remaining sterile. In these instances smears from the tissues three or four hours after the inoculation showed very extensive phagocytosis of the bacteria. Therefore, the process of immunization occurs as in a true vaccination, the organism reproducing itself in the tissues for probably 100 or 200 generations and its successive groups of receptors stimulating the production of corresponding groups of amboceptors in the animal body. It therefore is not difficult to understand why the immunity derived from vaccination in plague is greater than that obtained from the injection of the killed organism.

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[126] *This Journal* (1906), I, 181.

[127] *Jour. Exp. Med.* (1905), 7, 77.

Although abundant evidence had been obtained of the immunity produced in animals even more susceptible to plague infection than man (guinea pigs) by vaccination with these attenuated cultures, yet it was interesting to observe what evidence could be discovered, from a study of the blood serum, of the immunity resulting in human beings from such vaccinations. Therefore, the agglutinative reaction of the blood serum of twenty-six of the cases and the anti-infectious power in twenty-four were carefully studied. In order that the experiments might be carefully and completely performed, from 10 to 20 cubic centimeters of blood was collected from a vein, in each case under aseptic precautions; the blood being drawn ten days after vaccination and the reactions usually performed as soon as the serum had separated from the clot. The results of these experiments have already been recorded during the consideration of the agglutinative and anti-infectious properties of plague serum. The agglutinative reactions are also summarized in Table No. X (p. 252), and the anti-infectious ones in the series of inoculations on pages 275 to 280. From these tables it will be seen that the sera possess practically no agglutinating or anti-infectious power, at least in demonstrable amounts. Only in a few instances were there traces of a reaction. However, since in the serum of animals which had been demonstrated to be thoroughly immune to plague these substances (agglutinins and anti-infectious bodies) could not be demonstrated, we should hardly expect to find them in the blood serum of vaccinated human beings. Plague agglutinins and anti-infectious bodies only become developed in demonstrable amounts in animals that have been very highly immunized by large and repeated inoculations of the organism, as has already conclusively been demonstrated.

The opsonic power of the sera of the vaccinated individuals was next carefully investigated in eight instances, and this reaction proved to be a much more delicate and accurate means of demonstrating the existence of an immunity reaction following plague vaccination, than those already described. The experiments were performed with the strain "Pest Virulent" in the following manner:

The blood was collected 10 days after vaccination. 0.1 cubic centimeter of the serum, 0.1 cubic centimeter of a suspension of a normal guinea pig's washed leucocytes and 0.1 cubic centimeter of a suspension of the strain "Pest Virulent" were thoroughly mixed and the resulting suspension placed for thirty minutes at 37° C. Smears were then prepared and stained and 200 leucocytes counted. Control experiments of the nonphagocytic power of the washed leucocytes without serum were always prepared.

Series 62 (p. 267) shows the opsonic index both before and after vaccination. No attempt was made by frequent examination, to demonstrate a negative phase immediately following vaccination or to plot the curve of the reaction. As the table demonstrates, there was usually a distinct increase in the opsonic index following the inoculation, but this was not invariably the case. The increase in the opsonic action of the blood in animals immunized against plague has already been discussed under the subject of the immunizing action of plague immune sera. (See p. 265.)

The most satisfactory test of the demonstration of the immunity reaction in the blood of the vaccinated individuals was obtained from a study of the action of the serum in relation to the phenomenon of fixation of the complement after the method of Bordet and Gengou. These experiments have already been referred to under the discussion of the mechanism of the action of plague immune serum. Hæmolytic sera were first prepared by inoculating rabbits with goats' corpuscles; these were then collected from the rabbits, inactivated, and their hæmolytic power for a 5 per cent suspension of serum-free goat's corpuscles carefully determined. Fresh guinea pig serum was used to furnish the complement, and the minimum amount of complement for the reaction also ascertained. The human serum to be tested for plague immune bodies was then inactivated by heating for one hour at 56° C. A suspension in saline solution of a 24-hour agar culture of the virulent pest strain which was free of all clumps was employed. The experiments were performed in the following manner:

One cubic centimeter of the diluted human inactivated serum from the vaccinated person was added to 1 cubic centimeter of the suspension of bacteria and the unit dose of complement mixed with it. After one hour at 37° C., twice the dissolving dose of inactivated rabbits' serum specifically hæmolytic for goats' corpuscles and suspended in 1 cubic centimeter saline solution and 1 cubic centimeter of the 5 per cent solution of goats' corpuscles was added and the mixtures placed at 37° C. for 2 hours. At the end of this time the reactions were noted. All the necessary controls were also performed as may be seen from the tables where all the details of the reactions are given. The experiments were performed with the sera of four of the human beings and of two guinea pigs which had been vaccinated and with the serum of one plague immune horse. In each of these instances the deflection of the complement was obtained, demonstrating the presence of plague immune bodies in the serum tested. The results obtained are summarized in the following tables.

TABLE No. XI.—*Showing phenomenon of fixation of the complement with the serum of human beings and animals vaccinated against plague.*

[Saline suspension 0.85 per cent, q. s. to 4 cubic centimeters in each test tube.]

Inactivated rabbits' serum hæmolytic for goats' corpuscles.	5 per cent suspension goats' corpuscles.	Guinea pig's fresh serum for complement.	Vaccinated guinea pig serum (inactivated).		Vaccinated human serum No. I (inactivated).	Normal guinea pig serum (inactivated).	Normal human serum (inactivated).	Saline suspension of strain "Pest Virulent."	Hæmolysis.
			No. I.	No. II.					
0.0025	1	0.2	----------	----------	----------	----------	----------	----------	+
.005	1	.2	----------	----------	----------	----------	----------	----------	+
.005	1	.2	----------	----------	----------	----------	----------	1	+
.005	1	.2	0.1	----------	----------	----------	----------	----------	+
.005	1	.2	.025	----------	----------	----------	----------	----------	+
.005	1	.2	----------	0.05	----------	----------	----------	----------	+
.005	1	.2	----------	.025	----------	----------	----------	----------	+
.005	1	.2	----------	----------	0.1	----------	----------	----------	+
.005	1	.2	.1	----------	----------	----------	----------	1	0
.005	1	.2	.025	----------	----------	----------	----------	1	0
.005	1	.2	----------	.05	----------	----------	----------	1	0
.005	1	.2	----------	.025	----------	----------	----------	1	0
.005	1	.2	----------	----------	.1	----------	----------	1	0
.005	1	.2	----------	----------	----------	0.25	----------	1	+
.005	1	.2	----------	----------	----------	.1	----------	1	+
.005	1	.2	----------	----------	----------	.025	----------	1	+
.005	1	.2	----------	----------	----------	----------	0.25	1	+
.005	1	.2	----------	----------	----------	----------	.1	1	+
.005	1	.2	----------	----------	----------	.25	----------	----------	+
.005	1	.2	----------	----------	----------	.1	----------	----------	+
.005	1	.2	----------	----------	----------	.025	----------	----------	+
.005	1	.2	----------	----------	----------	----------	.25	----------	+
.005	1	.2	----------	----------	----------	----------	.1	----------	+
.005	1	----------	.1	----------	----------	----------	----------	1	0
.005	1	----------	.025	----------	----------	----------	----------	1	0
.005	1	----------	----------	.05	----------	----------	----------	1	0
.005	1	----------	----------	.025	----------	----------	----------	1	0
.005	1	----------	----------	----------	.1	----------	----------	1	0
.005	1	----------	----------	----------	----------	----------	----------	1	0
----------	1	.2	----------	----------	----------	----------	----------	----------	0
.005	1	----------	----------	----------	----------	----------	----------	----------	0
----------	1	----------	----------	----------	----------	----------	----------	----------	0

TABLE No. XII.—*Showing phenomenon of fixation of the complement with the serum of human beings vaccinated against plague and with horses' pest immune serum.*

[Saline suspension 0.85 per cent, q. s. to 4 cubic centimeters in each test tube.]

Inactivated rabbits' serum hæmolytic for goats' corpuscles.	5 per cent suspension goats' corpuscles.	Guinea pig's fresh serum for complement.	Horses' pest immune serum (inactivated).	Vaccinated human serum (inactivated). No. II.	No. III.	No. IV.	Normal horses' serum (inactivated).	Normal human serum (inactivated).	Saline suspension of strain "Pest Virulent."	Hæmolysis.
0.0016	1	0.15	--------	--------	--------	--------	--------	--------	--------	+
.0033	1	.15	--------	--------	--------	--------	--------	--------	--------	+
.0033	1	.15	--------	--------	--------	--------	--------	--------	1	+
.0033	1	.15	0.02	--------	--------	--------	--------	--------	--------	+
.0033	1	.15	.01	--------	--------	--------	--------	--------	--------	+
.0033	1	.15	--------	0.1	--------	--------	--------	--------	--------	+
.0033	1	.15	--------	--------	0.1	--------	--------	--------	--------	+
.0033	1	.15	--------	--------	.05	--------	--------	--------	--------	+
.0033	1	.15	--------	--------	--------	0.05	--------	--------	--------	+
.0033	1	.15	--------	--------	--------	.033	--------	--------	--------	+
.0033	1	.15	.02	--------	--------	--------	--------	--------	1	0
.0033	1	.15	.01	--------	--------	--------	--------	--------	1	0
.0033	1	.15	--------	.1	--------	--------	--------	--------	1	0
.0033	1	.15	--------	--------	.1	--------	--------	--------	1	0
.0033	1	.15	--------	--------	.05	--------	--------	--------	1	0
.0033	1	.15	--------	--------	--------	.05	--------	--------	1	0
.0033	1	.15	--------	--------	--------	.033	--------	--------	1	0
.0033	1	.15	--------	--------	--------	--------	0.05	--------	1	+
.0033	1	.15	--------	--------	--------	--------	.01	--------	1	+
.0033	1	.15	--------	--------	--------	--------	--------	0.1	1	+
.0033	1	.15	--------	--------	--------	--------	--------	.05	1	+
.0033	1	.15	--------	--------	--------	--------	.05	--------	--------	+
.0033	1	.15	--------	--------	--------	--------	.01	--------	--------	+
.0033	1	.15	--------	--------	--------	--------	--------	.1	--------	+
.0033	1	.15	--------	--------	--------	--------	--------	.05	--------	+
.0033	1	--------	.02	--------	--------	--------	--------	--------	1	0
.0033	1	--------	.01	--------	--------	--------	--------	--------	1	0
.0033	1	--------	--------	.1	--------	--------	--------	--------	1	0
.0033	1	--------	--------	--------	.1	--------	--------	--------	1	0
.0033	1	--------	--------	--------	.05	--------	--------	--------	1	0
.0033	1	--------	--------	--------	--------	.05	--------	--------	1	0
.0033	1	--------	--------	--------	--------	.033	--------	--------	1	0
.0033	1	--------	--------	--------	--------	--------	--------	--------	1	0
--------	1	.15	--------	--------	--------	--------	--------	--------	--------	0
.0033	1	--------	--------	--------	--------	--------	--------	--------	--------	0
--------	1	--------	--------	--------	--------	--------	--------	--------	--------	0

Therefore, following plague vaccination in human beings, an immunity reaction occurs which consists in the development of anti-bodies thrown off into the circulation and whose presence may be demonstrated both by the opsonic reaction of Wright and also by the method of the fixation of the complement of Bordet and Gengou.

Since my first report upon the method of vaccination against plague in man in 1905, such inoculations have been made from time to time and during the past year nearly 200 vaccinations have been performed. The cultures with which I have worked have proven themselves to be entirely safe for human beings[128] and I have observed no unfavorable results in the inoculated. However, as emphasized in my previous communication, vaccinations against plague should not be made in man unless the investigator can guarantee the particular organism with which he is working to be of sufficient attenuation to be no longer dangerous for human beings. Strains of the bacilli which invariably no longer kill guinea pigs of 250 grams weight upon subcutaneous inoculation in amounts of one agar slant culture are probably safe in small amounts for human beings. Owing to the great stability in virulence of the plague bacillus under certain conditions, its use in vaccination is much simplified.

During the past year and a half I have been able to detect no change either in virulence, toxicity, or immunizing power in the strains with which I have performed my vaccinations in man and in animals. The cultures apparently possess the same immunizing power to-day as they did at the commencement of the experiments. However, it is very probable that they could be further attenuated by the artificial means previously described.

Douglas and W. Bullock[129] (whose article on plague has just appeared in Allbutt and Rolleston's System of Medicine as my article goes to press) in commenting upon my work in vaccination against plague make the following statement:

> Naturally very great care would be necessary in recommending a method like this on a big scale in plague stricken communities, as from unforeseen circumstances the virulence might increase and plague be induced.

As I have pointed out, there is no evidence to support this statement, and my cultures which for nearly two years have been used at intervals in human beings are as safe for use in man to-day as they were at the time of my first inoculations.

[128] The accident in relation to some inoculations which recently occurred in Bilibid Prison in Manila and about which a report will shortly be made, was not due to these plague vaccinations.

[129] Allbutt and Rolleston: System of Medicine (1907), 2, part 2, 408.

XVI. CONCLUSION.

In recommending for man vaccination against plague with suitable cultures I wish to emphasize the fact that the method is not infallible and that very brilliant results may not always be obtained by it. In the vaccination of large numbers of human beings, owing to individual variations in susceptibility to plague infection and natural resistance, just as in the experiments described in monkeys, certainly all of the individuals will not be protected against lethal infection by a single vaccination with one agar culture, but a certain proportion of them may be immunized by this method and an appreciable degree of immunity may be retained for at least nine months. The population in a plague stricken district may gradually be immunized against this disease by the employment of vaccination.

In plague, as in smallpox vaccinations, it may frequently be necessary to repeat the vaccination and perhaps with a larger dose, in order to secure a satisfactory immunity.

As yet we know of no practical method for the detection of those cases which would require a second plague vaccination to produce in them a sufficient immunity to protect them from the natural manner of infection of the disease. It is perhaps possible that by a careful study of the opsonic index in man and animals both before and after vaccination and with subsequent attempts to infect the animal in order to observe the degree of immunization which has resulted, a standard may be obtained by means of which we may be able to judge from the amount of the increase in the index whether the individual has acquired a satisfactory immunity from the first vaccination.

Owing to the complexity of the experiments necessary for the determination of the phenomenon of the fixation of the complement in the serum of the vaccinated, this method will probably never assume practical value for the determination in a large number of cases of the degree of plague immunity acquired.

Professor Kitasato in a recent address published in a previous number of this JOURNAL [130] made the statement that:

> Plague is not only objectionable to the people of a locality but it is an enemy to mankind in general. All civilized nations must fight this common enemy. I believe there should be an international conference to discuss a plan to collect money and to organize an international army to combat and vanquish this disease wherever it appears. Expeditions should be sent to the interior regions

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[130] *This Journal* (1906), 1, 465.

of India and South China. The cost of such an enterprise would be only a small part of what the civilized nations are constantly expending in keeping armies and navies. Even the amount which every country is spending for the prevention of the pestilence would suffice. My suggestion only lacks a leader, and I see that the United States, one of the greatest nations of the earth, has such a leader in the person of its president, Theodore Roosevelt, who has already done so much for humanity and whose noble works are being admired by the whole world.

It is obvious that sufficient attention is not being given to combating this disease in its endemic centers and from which there is continued danger of invasion by the pestilence into other countries. This fact is conclusively demonstrated because the disease is not markedly decreasing in these centers. Indeed it would appear that India is at present suffering from an epidemic of plague equal to the one which occurred there in 1904. The official monthly returns from that country for the present year (1907) show 58,438 deaths from plague in January, 98,397 in February and 171,522 in March.[131]

During the past year the British Indian Plague Commission, under the direction of Martin and Lamb, have by their very important studies thrown much light upon the question of the manner of transmission of the disease. Frequent reference has been made in the present article to their researches as well as to the very valuable ones of Kolle and his pupils in Berlin, and it is hoped that the laboratory investigations of these observers together with the studies of Kitasato in Japan will serve further to stimulate more active work in the extermination of this pestilence.

From my investigations it would appear that general vaccination in the endemic centers will be a valuable means of assistance in accomplishing this end.

[131] *Lancet.* (1907), 1, 1200.

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B. MEDICAL SCIENCES

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NECATOR AMERICANUS IN NATIVES OF THE PHILIPPINE ISLANDS.[1]

By CLARENCE L. COLE.[2]

In the routine examinations of patients admitted to the United States Army Hospital at Manila, P. I., many members of Philippine Scout organizations and native civilian employees have been found to have a few ova of *Uncinaria* in their stools. In these cases the presence of *Uncinaria* has not made itself manifest clinically to such an extent that a diagnosis of uncinariasis has often been made, and as many of these patients have been admitted from temporary stations in the field, where microscopical examinations could not be undertaken, they were therefore sent to the hospital accompanied by a different diagnosis. It has been customary to accept *Uncinaria* ova as belonging to the Old World species, without studying the adult parasites obtained after the administration of vermicides to those patients whose stools have shown ova, because of the fact that no other varieties of *Uncinaria* have been reported from the Philippine Islands.

In September, 1906, a private of the Twenty-eighth Company, Philippine Scouts, was admitted to the Division Hospital, Manila, P. I., and ova of *Uncinaria* were found in his stool, they being more abundant in this case than in any other which had been examined in the preceding six months. Three or four ova could be observed in the field at one time when the $\frac{2}{3}$ objective was used. A very few *Uncinaria* were recovered in the first stools after the administration of thymol; two days later, following a course of thymol, about ninety were obtained and ova were not found again until fifteen days had elapsed, when, after a third course of the drug a large number of *Uncinaria* (possibly fifty) were again recovered. No more ova were encountered in the stools of this patient. The *Uncinaria* obtained from this case corresponded quite closely in measurements to those given by Stiles[3] for the variety of *Uncinaria* described by him and to which he has given the name *Uncinaria americana* (*Necator americanus*).[4] When examining the female of this variety the position of the vulva was noticed as one of the first things differentiating *Necator* from *Agchylostoma*. In all the specimens examined it has been found to be near the anterior one-third of the body. (Pl. I, figs. 1 and 2.) From these facts it appears that the specimens are *Necator americanus*. In the meantime, similar *Uncinaria* had been recovered from other patients. The medical publications reaching Manila during the progress of this work established the fact that *Necator americanus* had been identified in material from Guam and China.[5]

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[1] Read at the Fourth Annual Meeting of the Philippine Islands Medical Association, Manila, February 28, 1907, with the permission of the chief surgeon, Philippines Division, Manila, P. I.

[2] First lieutenant and assistant surgeon, United States Army; pathologist and bacteriologist, United States Army, Division Hospital, Manila.

As interest had been aroused by the supposed finding of *Necator americanus* in natives of the Philippine Islands, great care was taken to secure as much material as possible for comparison. In addition to the patient mentioned above, seven other Filipino and two American soldiers have been found to harbor this parasite. The stations of these soldiers were widely separated and in different islands of the Archipelago as follows: four from the same station as the case just referred to, two from the Island of Samar, another from the city of Manila, one American soldier was sent to this hospital from the Island of Guimaras, the other from Camp Daraga, Luzon. No less than five hundred *Uncinaria* have been collected from these cases.[6]

The structures which are constantly present and unmistakable in these *Uncinaria,* which resemble *Necator americanus* more than they do any other of the *Strongyloidæ,* were thought to be of sufficient importance to form the basis for the supposition that a new species of *Necator* had been found in the Philippine Islands.

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[3] *P. H. & M. H. S. Bull.* (1903), No. 10.

[4] A Young Stage of the American Hookworm—*Necator Americanus* (Stiles 1902)—8 to 12 Days after Skin Infection in Rabbits and Dogs. Stiles and Goldberger, *Am. Med.* (1906), **11**, 63.

[5] *Bull. Johns Hopkins Hosp.* (1906), **17**, 313.

[6] Photomicrographs and camera-lucida drawings have been made of a number of specimens, to assist in demonstrating the presence of the newly described organs not found in the descriptions or illustrations by Stiles; whereas the apparently new organs, or their homologues, were illustrated by the same author in *Agchylostoma duodenale.* (After Schulthess (copied from Blanchard).)

About the time this conclusion had been reached and an effort had been made to describe this supposedly new variety of *Uncinaria,* it appeared that others were finding species of *Necator* differing from *Necator americanus.*

Stephens[7] thinks that *Necator americanus* may be only one of many species which exist in different parts of the world. He has found varieties of *Necator* differing from *Necator americanus* in material collected in Burma and Assam. Loos[8] of Cairo confirmed Stephens's statement and identified at least three different species in the material which the latter had collected. It is not known upon what characteristics Stephens bases his differentiation as no specimens have been described by him.

In January, 1907, through the kindness of Lieut. P. E. Garrison, United States Navy, medical zoölogist at the Bureau of Science, an opportunity was given to compare the *Uncinaria* I had obtained with type specimens of *Necator americanus* which he had procured in Washington from Stiles. They were found to be similar.[9]

After quoting the description given by Stiles for *Necator americanus* I will attempt to demonstrate by means of drawings and photomicrographs the characteristics of the *Uncinaria* found here.

The New World hookworm of man, *Uncinaria americana* Stiles, 1902.

Specific diagnosis.—Uncinaria: Body cylindrical, somewhat attenuated anteriorly. Buccal capsule with a ventral pair of prominent semilunar plates or lips, similar to *U. stenocephala,* and a dorsal pair of slightly developed lips of the same nature; dorsal conical median tooth projects prominently into the buccal cavity, similar to *Monodontus;* one pair of dorsal and one pair of ventral submedian lancets deep in buccal capsule. Male, 7 to 9 millimeters long; caudal bursa with short, dorsomedian lobe, which often appears as if it were divided into two lobes, and with prominent lateral lobes united ventrally by an indistinct ventral lobe; common base of dorsal and dorso-lateral rays very short; dorsal ray divided to its base, its two branches being prominently divergent and their tips being bipartite; spicules long and slender. Female, 9 to 11 millimeters long; vulva in anterior half of the body, but near equator. Eggs, ellipsoid, 64 to 76 μ long by 36 to 40 μ broad, in some cases partially segmented in utero, in other (rare) cases containing a fully developed embryo when oviposited.

Habitat.—Small intestine of man (*Homo sapiens*) in America (determined to date (1902) for Virginia, North and South Carolina, Georgia, Florida, Alabama, Texas, Porto Rico, Cuba, and Brazil).

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[7] *Indian Med. Gaz.* (1906), **41**, 398.

[8] *Ibid.*

[9] The single male specimen of *Necator americanus* which was given me had been mounted for some time, and as a result of the pressure of the cover glass the specimen is flattened and can not be placed in as favorable position for demonstrating the structures in question as some of the fresh ones from the Philippines.

The following characteristics may be distinguished in all the *Uncinaria* recovered from the cases here reported.

A slight depression in the cuticular layer of the body wall upon the ventral surface 0.5 millimeter posterior to the oral margin. (See Pl. I, figs. 1 to 4.) At the center of this depression there is an opening into the body cavity. This corresponds to the position of the excretory pore in *Uncinaria duodenalis*. In many specimens this opening is surrounded by a mass of granular matter which can easily be removed with no disturbance of the cuticle at the site of attachment. The granular material may be seen within the mouth of the pore in some of the specimens, apparently being extruded from the body. The cervical papillæ are situated upon either side of the excretory pore. The position of the excretory pore is not indicated in the original drawings of *Necator americanus*.

The specimens collected here do not correspond exactly in size with those collected in America. The extreme measurements for the male specimens are from 7.25 millimeters in length for the shortest found, to 8.5 millimeters for the longest; the shortest female measured 10 millimeters in length, the longest 13.5 millimeters.

The presence of a pair of small, ray-like organs within the caudal bursa of the male (not mentioned in the original description), the constant presence of an enlargement of the bursa adjacent to them, the shape of the dorso-lateral rays and the prominence of the dorsal and ventral lobes of the caudal bursa attract one's attention.

The caudal bursa of the male consists of two lateral lobes continuous with small dorsal leaflets. Each of the leaflets partly surrounds one of the dorsal rays. (See Pl. II.) Ventrally the bursa presents a slight enlargement just posterior to the point at which it is continuous with the body wall. (Pl. II.) The lateral lobes are connected ventrally by a delicate, filmy ventral lobe. (Pl. II, figs. 3 and 4.) When the lateral lobes are closely approximated the ventral lobe may frequently be seen folded upon itself with the fold deep between the lateral bursa. (Pl. II, fig. 3.)

The dorso-lateral rays are long, slender and clavate. (Pl. II, figs. 3 and 4; Pl. IV, fig. 1.)

Within the bursa, anterior to the ventral rays, a pair of small organs may be seen resembling rays in structure, but more delicate. They are constant in all males. It is rarely possible to see more than one in the same focus, but the specimen may be rotated under the cover glass until both organs may clearly be distinguished. These organs are homologous with the praecaudal papillæ of *Uncinaria duodenalis*. They arise from the large trunks which give origin to the lateral, ventro-lateral, and ventral rays of their respective sides, but anterior to these. By placing the specimen in the position that shows the dorsal rays most clearly, one or perhaps both of the smaller rays may be seen directly opposite, upon the ventral surface, the tips of the rays projecting into the ventral enlargement of the caudal bursa. (Pl. I, fig. 3; Pl. II; Pl. III, fig. 1.)

The spicules terminate in barbed tips and the length of the free ends of the spicules is not constant. Some may terminate within the caudal bursa while others protrude as much as one-half the length of the caudal bursa beyond it. (Pl. II, figs. 3 and 4.)

The following measurements of three female worms illustrate the relation of the distance from the head to the vulva to the total length of the worm:

No. 1: Length of worm, 11.6 millimeters; distance from head to vulva, 5 millimeters. No. 2: Length of worm, 10.7 millimeters; distance from head to vulva, 4.4 millimeters. No. 3: Length of worm, 12.6 millimeters; distance from head to vulva, 5.5 millimeters.

The ratio which the length from head to vulva bears to the length from the vulva to the tip of the tail in the three cases above given is as follows:

No. 1, 1: 2.32. No. 2, 1: 2.43. No. 3, 1: 2.29.

The ova are larger than those of *Uncinaria duodenalis.* (Pl. IV, fig. 2.) Harris[10] reports the measurements of ova of *Necator americanus* from 57.5 to 80 μ in length and 35 to 52.5 μ in breadth; and the average from a large number of ova as 66.52 μ in length by 42.53 μ in breadth. In these cases the ova measured from 58 to 73 μ in length by 36 to 46 μ in breadth. The average measurement of 40 ova was 66 by 40 μ.

The attention of the commanding officer of the Division Hospital, Major F. J. Ives, United States Army, having been invited to the number of patients from the Twenty-eighth Company of Philippine Scouts admitted to the hospital and infected with *Necator americanus,* he requested the chief surgeon of the Philippines Division to authorize an investigation. The result was that *Uncinaria* ova were found in the first slide from 13 of 25 men (52 per cent) examined, who at that time were on duty.

The fact that *Necator americanus* has been recovered from the stools of all enlisted men showing *Uncinaria* ova, admitted to this hospital since September, 1906, and that ova corresponding in measurements to those of *Necator americanus* have been found in the stools of 52 per cent of the men examined in one company, would lead one to think that a large percentage of natives of the Philippine Islands harbor this parasite. Craig[11] reported cases of uncinariasis in soldiers who had returned from the Philippine Islands to the United States, but in those only *Uncinaria duodenalis* was found.

As both varieties of *Uncinaria* are present in the Philippine Islands and as these investigations demonstrate that *Necator americanus* is quite prevalent, uncinariasis would appear to be one of the most common diseases to be found here and it would be expected that many severe cases would be encountered.

Seventy-six officers and men of the Regular Army have been treated for uncinariasis in the Division Hospital since its organization in 1898, and of this number only 38 were admitted with a diagnosis of uncinariasis or ankylostomiasis. The histories of these 38 patients show gastro-intestinal disturbance in 32, anæmia in 6, heart murmurs in 2. Dyspnœa and œdema were not noted among the symptoms in any of them. Six cases were uncomplicated by other diseases; 21 were suffering with dysentery as well as uncinariasis; 5 with diarrhœa; 5 with malaria; 1

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[10] Uncinariasis (Ankylostomiasis); Its Frequency and Importance in the Southern States. *The Atlanta Journ.—Record of Medicine* (1903).

[11] The Occurrence of Uncinariasis (Ankylostomiasis) in Soldiers of the United States Army. *Am. J. Med. Sci.* (1903), **126**, 798.

with sprue; 3 with filariasis; 1 with rheumatism; 1 with liver abscess, and 1 with epilepsy.

Thirty-eight cases have been admitted to the hospital for other conditions, and ova of *Uncinaria* have subsequently been found in their stools. The diagnosis in nineteen of these has been changed to uncinariasis with no complication. In nearly all of the others gastro-intestinal disturbance was marked; anæmia being observed in five. Of the remaining cases, dysentery, tuberculosis and malaria were common complications. Three of these were diagnosed as beriberi and in one the diagnosis was changed to uncinariasis.

I have not had an opportunity during my tour of duty in these Islands to travel through the country districts where one would expect to find conditions most favorable for the development of *Uncinaria* and to encounter the most serious cases of uncinariasis. The patients I have had an opportunity to examine and the records of cases to which I have been able to refer, have all been of soldiers who had not more than three years previously passed a rigid physical examination. These men had only been exposed to the infection during longer or shorter periods of active service in the field, but some of the Scouts may have been suffering from mild infections of a number of years' duration.

In the United States it has been found that women and children are the greatest sufferers, except in the cases where the employment of the men caused an exposure of the bare skin to the contaminated earth. Soldiers are exposed to infection by this parasite while performing field service and then only during the rainy season. It would hardly be possible that cases as severe would be found among these picked men as among natives in a barrio.

The health of the soldier in garrison is safeguarded by all the precautions known to the sanitarian; it would be a grave condition indeed if under these circumstances severe cases of uncinariasis could develop at all and it could hardly be possible for a severe infection to occur in any great number of soldiers during the short tours of field duty which are customary in these Islands.

In the provinces no precautions at all are taken properly to dispose of the waste. Privies and vaults are among the rarities and the surface of the ground around by far the greater majority of the houses has been contaminated from the time that the houses were built. The abundant growth of vegetation around dwellings furnishes the most favorable conditions (shade and moisture) for preserving the vitality of rhabditiform embryos, and the barefooted householder and his family are constantly exposed to the infection.

I wish to quote a few lines from Ashford[12] in regard to the severity of this disease in Porto Rico:

> There seems to be no doubt that for many years from 5,000 to 7,000 people have been dying annually from this easily curable and preventable disease; nor is it an exaggeration to say that the great majority of those affected with *Necator americanus* are physically incapable of rendering anything like their full quota of labor. * * * Fully 70 per cent of the worm carriers suffer more or less severely from their infection, and an incredibly large number of men that should form the bone and sinew of this healthful and beautiful island are ghost-like invalids, compelled to work, though sick, that their families may not starve.

If such a condition exists in Porto Rico why should not a similar one be found in the Philippine Islands where we have the same parasite, all the favoring conditions for its growth which a tropical climate affords and a population whose habits are such that the greatest care could not facilitate the dissemination of the infection more perfectly?

There are two conditions existing in Porto Rico which have been found favorable for the spread of uncinariasis that are dissimilar in these Islands. They are:

First. An occupation in which a large number of barefooted people are gathered together during the rainy season, and each by his careless habits increases the danger to the others.

Second. The Porto Ricans are a lighter skinned race than the Filipinos.

In the Philippine Islands the cultivation of coffee is not a very extensive industry and the number of people engaged in it is comparatively small. There is no single industry in which great numbers of the Filipinos are engaged that presents equally favorable opportunities for universal dissemination of the infection. Nearly every writer upon uncinariasis states that the Negro living in localities where uncinariasis is prevalent does not suffer from the anæmia which lighter skinned people are subject to. The Filipino being quite dark skinned may be less susceptible than the Porto Rican.

For many years authorities have considered the Porto Rican anæmia to be due to an innutritious diet. The Porto Rican Anæmia Commission proved the fallacy of this supposition by restoring thousands to health by removing the *Uncinaria* with which the poorer people were infected. One would not expect the poor negro of our Southern States or of Porto Rico to subsist upon a more nutritious diet than the poor white of the same locality; yet many investigators have observed the relative infrequency of severe cases of anæmia in the negroes of these districts.

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[12] *Military Surg.* (1907), **20**, 41.

CONCLUSION.

The points which I wish to emphasize as a result of these investigations may be summarized as follows:

Necator americanus is a common parasite in natives of the Philippine Islands.

This infection occasions a great loss of time to the Government through illness of the enlisted men, principally from the Scout organizations.

There is an unnecessary increase of expense for medicines and hospital supplies because of this condition.

Every man infected with *Uncinaria* is a menace to his comrades and a source of danger to the community in which he is stationed.

No person suffering from uncinariasis, mild or severe, is capable of performing his duty as efficiently as one who is free from this infection.

Everyone suffering from uncinariasis, although it may be mild, is more susceptible to other diseases and having contracted a complicating disease, is more severely attacked because of his weakened condition and also his period of illness is necessarily longer.

Uncinariasis is a disease which yields readily to treatment, with little danger and with positive results. If uncinariasis is as prevalent in the Philippine Islands as the few examinations here reported would indicate, then there is no way in which the systematic expenditure of a small amount of money will bring a greater return in health, happiness, efficiency, and increased prosperity, than in its eradication.

ILLUSTRATIONS.

[*Photomicrographs by Mr. Charles Martin, photographer of Bureau of Science, Manila.*]

PLATE I.

FIG. 1. Female *Necator americanus*. (Original.) *A*, the vulva; *B*, the excretory pore; *C*, the anus.

2. Photomicrograph of female *Necator americanus*. Magnification, 14×1. *A*, excretory pore; *B*, vulva; *C*, anus.

3. Male *Necator americanus*. (Original.) *A*, excretory pore; *B*, *C*, dorsal rays; *D*, one of the precaudal papillæ.

4. High magnification of the excretory pore, *Necator americanus*. 400×1. (Original.)

PLATE II.

FIG. 1. Outline drawing of the tail of the male showing both precaudal papillæ, *Necator americanus*. (Original.)

2. Tail of the male, *Necator americanus*. (Original.) *A*, dorsal rays; *B*, dorsal leaflets of the lateral lobes of the caudal bursa; *C*, one of the precaudal papillæ; *D*, ventral enlargement of the caudal bursa; *E*, ventral lobe of the caudal bursa.

3. Illustration of the tail of the male *Necator americanus* showing the principal structures. (Original.) *A*, dorsal rays; *B*, dorso-lateral ray; *C*, lateral rays; *D*, ventro-lateral rays; *E*, spicules; *F*, ventral rays; *G*, ventral lobe of the caudal bursa; *H*, precaudal papilla; *I*, ventral prominence of caudal bursa.

4. Photomicrograph of tail of male *Necator americanus*. Magnification, 87×1. *A*, dorsal rays; *B*, precaudal papilla; *D*, the ventral lobe of the caudal bursa; *J*, barbed tips of the spicules; *K*, dorsal leaflets of lateral lobes of the caudal bursa; *R*, the ventral enlargement of the caudal bursa adjacent to the precaudal papillæ.

PLATE III.

FIG. 1. Photomicrograph showing precaudal papillæ of the specimen of *Necator americanus* given me by Dr. Garrison. Magnification, 106×1. *A*, precaudal papillæ; *B*, ventral enlargement of the caudal bursa adjacent to the papillæ.

2. Photomicrograph of male *Necator americanus*. Magnification, 10×1.

3. Outline drawing showing the ventral lobe of the caudal bursa folded between the lateral lobes, *Necator americanus*. (Original.)

4. Illustration of the tail of the male *Necator americanus* dorso-ventral position. *A*, dorsal rays; *B*, dorso-lateral rays; *C*, lateral rays; *D*, ventro-lateral rays; *E*, ventral rays; *F*, spicules. The precaudal papillæ are too delicate to be seen through the thick body.

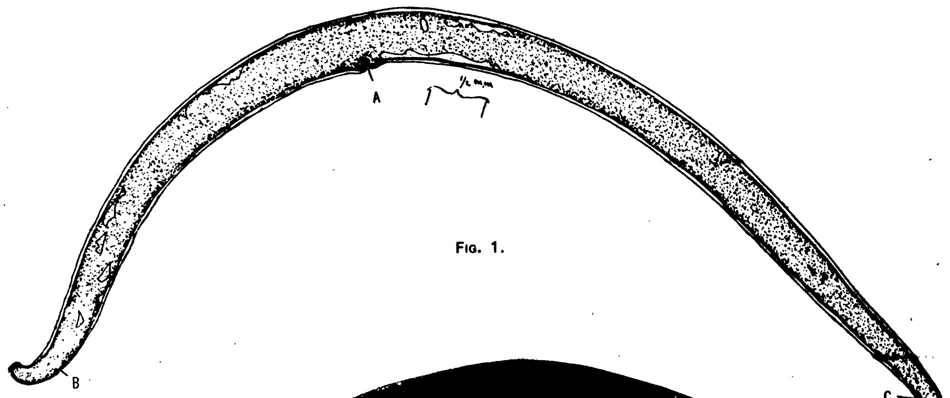
PLATE IV.

FIG. 1. Photomicrograph of tail of male *Necator americanus*, postero-ventrally. The same specimen illustrated by drawing. Magnification, 56×1.

2. Illustration of ten uncinaria ova in different stages of segmentation with their respective measurements. (Original.) *1*, 72×40 μ; *2*, 62×38 μ; *3*, 58×42 μ; *4*, 73×42 μ; *5*, 58×43 μ; *6*, 68×36 μ; *7*, 68×38 μ; *8*, 62×38 μ; *9*, 68×38 μ; *10*, 62×42 μ.

3. Photomicrograph of buccal plates in mouth of *Necator americanus*. Magnification, 46×1. *A*, large ventral plates; *B*, dorsal plates.

4. Tail of the female greatly enlarged, *Necator americanus*. (Original.) *A*, anus.



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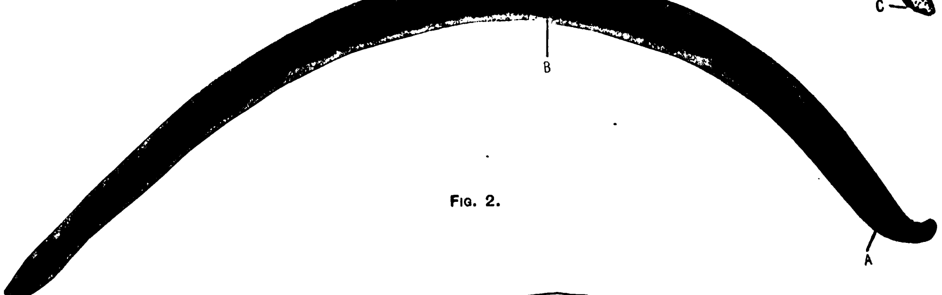

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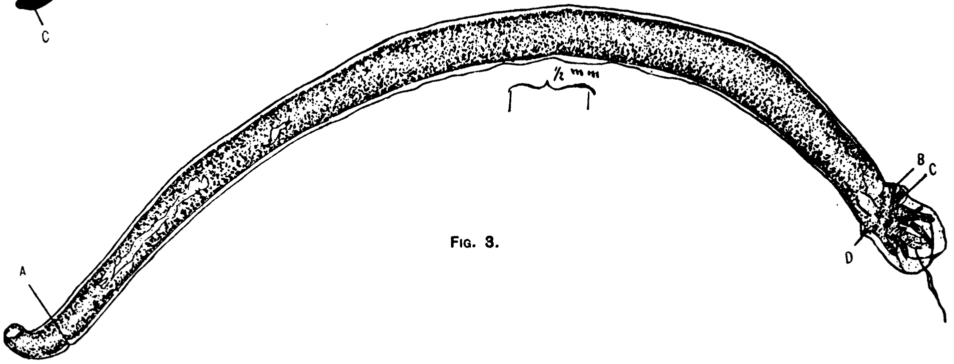

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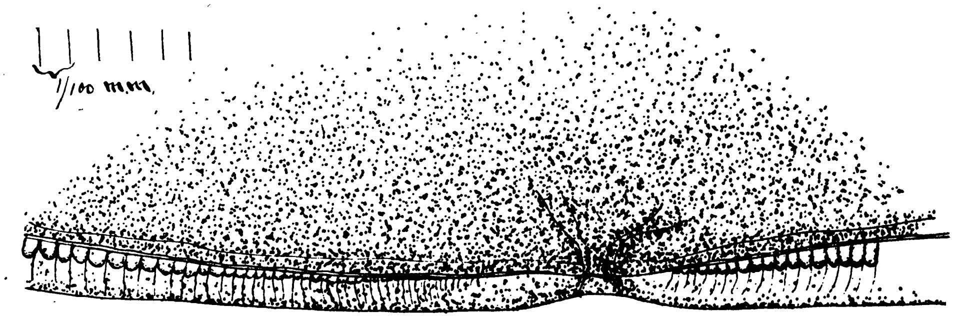

FIG. 4.

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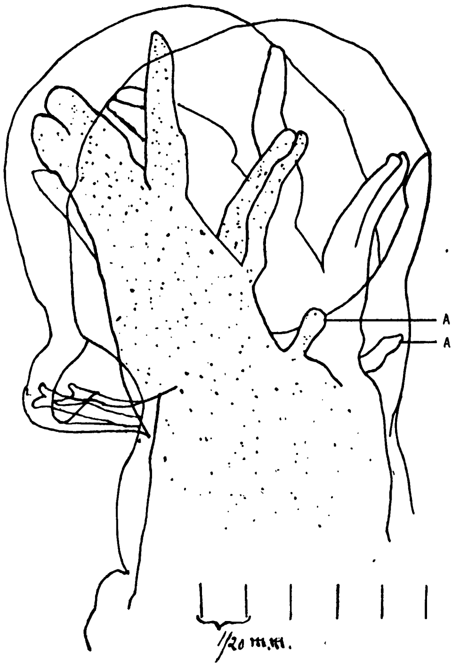

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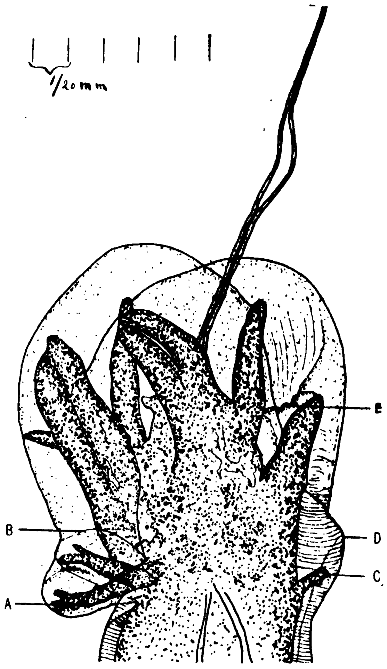

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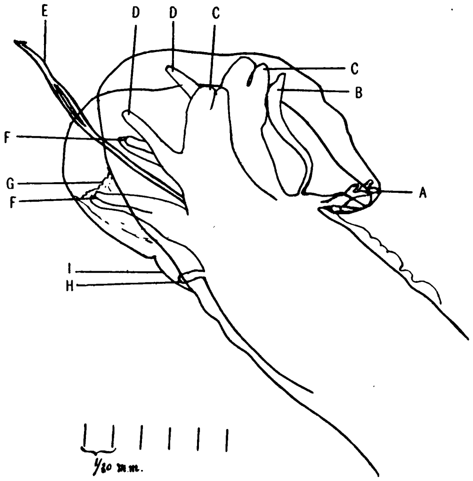

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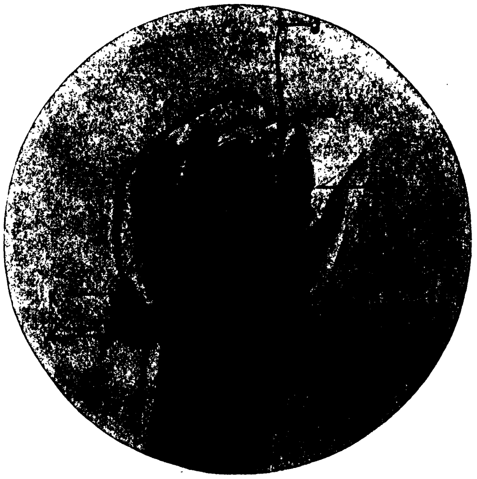

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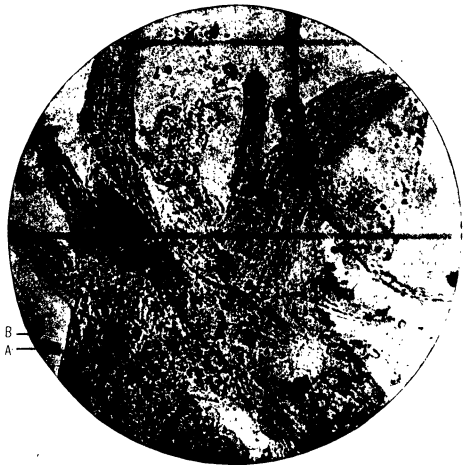

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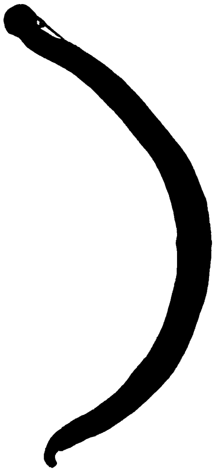

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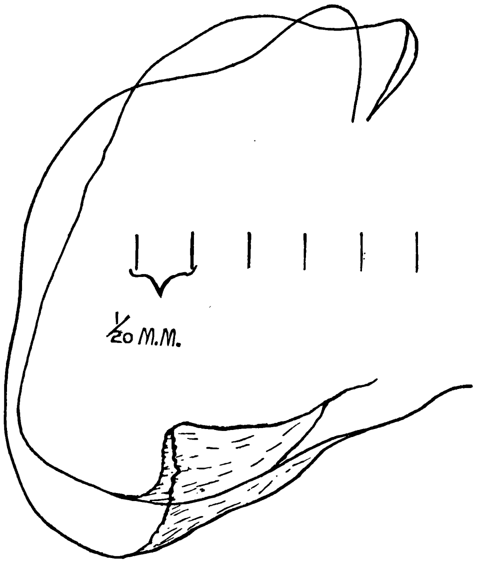

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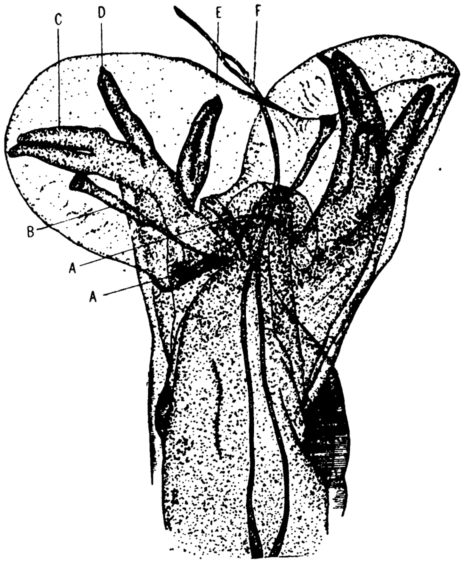
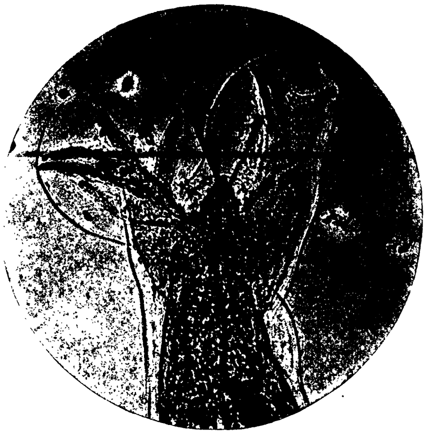


FIG. 4.

PLATE III.



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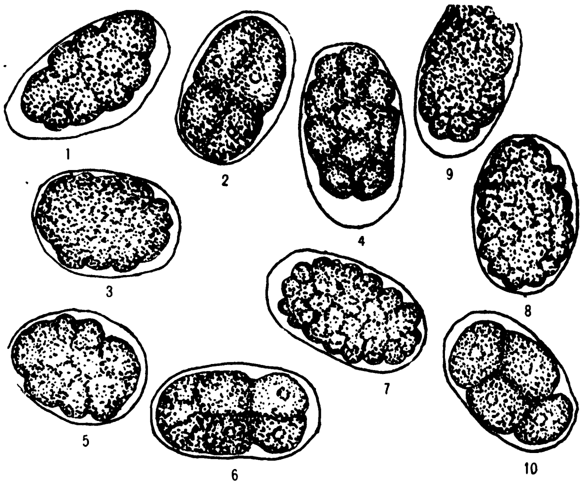

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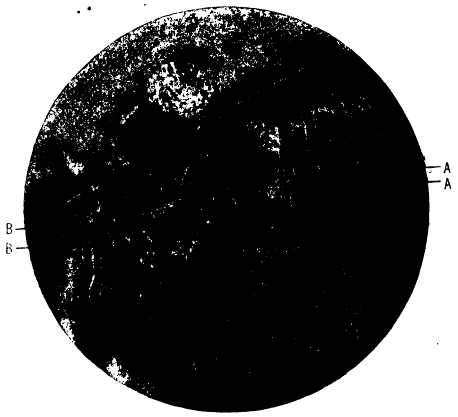

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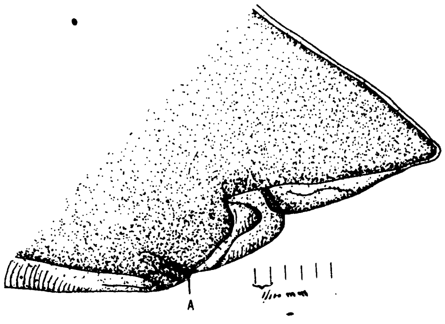

FIG. 4.

PLATE IV.

THE RECENT TREND OF IMMUNITY RESEARCH.[1]

By Harry T. Marshall.
(*From the Biological Laboratory of the Bureau of Science, Manila, P. I.*)

The term immunity has come to have two meanings within recent years; it retains its older significance and it is also used broadly to designate the condition resulting when an organism has reacted to foreign albuminous material. Researches along the more general lines of immunity have led in such widely divergent directions that a general review of the literature can not be attempted within the limits of the time allotted to this paper. Therefore, I have selected for review two subjects, which possibly are somewhat fresher than the others and which are commanding considerable attention at present. We will consider first the phenomenon known variously as complement deflection, complement deviation, complement diversion or blocking of complement, and second, Bail's aggressin hypothesis of infection and immunity.

I. DEFLECTION OF COMPLEMENT.

Early in the course of Bordet's(3) and Ehrlich's(8) studies on the mechanism of hæmolysis, it was found to be possible to prevent by certain fairly definite procedures the union of complement with red corpuscles previously laden with immune body. The bearing of this phenomenon upon the receptor theory and its value as a demonstration of the receptor explanation of hæmolysis was discussed, and for the sake of simplicity the property possessed by a fluid of blocking the action of complement was attributed to a hypothetical substance termed "anti-complement." Subsequently, what appeared to be another type of the blocking occurring during bacteriolysis, was described by Neisser and Wechsberg(29).

We must remember that it never has been possible to obtain complement pure, and that it is defined only in part, the definition at present demanding (*a*) that complement shall be capable of uniting with attached amboceptors by the hypothetical haptophore group, and (*b*) that it shall, either by its own inherent energy, or by its mere presence, bring about destructive changes in the body to which it unites (red corpuscles, bacterium, etc.). These changes are tentatively described as being of a fermentative or enzyme-like nature. The instability of complement is a more or less permanent characteristic, and equally characteristic are the facts that complement is normally present in varying amount in fresh serum, and that the quantity can not be influenced by the usual processes of immunization, provided the health of the animal remains unimpaired. If either of the first two characteristics fails, we have at present no means at our disposal of recognizing complement. A number of convincing arguments have been brought forward to prove that there are different complements in different serums, and also that any one specimen of serum contains a number of complements, although other observers claim that all forms of complement action are due to a single substance or form of energy in the serum. Whether complement has other actions we do not know, nor whether it is capable of uniting with a variety of other substances. Similarly, we do not know anything of anti-complement except the one fact that it prevents the action of complement. Whether a variety of substances or conditions could be the cause of such an inhibition was not determined at the time that anti-complements were first considered. Bordet (4) claims that specific anti-complements can be produced, the action of which is different from that occurring in complement deflection, and Marshall and Morgenroth (20) found it possible with a nonspecific anti-complement to block out certain definite complement activities from a serum without interfering with its other complement actions. On the other hand Pfeiffer and his colleagues (10:30) claim that the phenomenon of complement deflection explains reactions hitherto accounted for as the result of anti-amboceptor action.

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[1] Read at the Fourth Annual Meeting of the Philippine Islands Medical Association, Manila, March 2, 1907.

The phenomenon of complement deflection was first described in 1901 by Bordet and Gengou (5) who found that hæmolysis was no longer produced in properly prepared corpuscles if the complement-containing fresh serum was previously added to a mixture of bacterial emulsion and specific anti-bacterial serum.

In 1905, Gay (11, 12, 13), in Bordet's laboratory, discussed this phenomenon, and concluded that the complement was removed by the union of specific precipitin with precipitable substance. He found, as Sachs (32) did also, that the immune body was not affected in this reaction, only the complement being removed. He attributed the well-known Neisser-Wechsberg phenomenon of deflection and the antilytic action of normal serum described by Pfeiffer and Friedberger, and the anti-complementary action of normal serum described by Sachs, to the same phenomenon of precipitation. He holds that the above-mentioned investigators overlooked minute traces of precipitable substance which remained attached to the corpuscles, etc., after one washing with salt solution, and which could be removed only by repeated washings.

Moreschi (22, 23), working independently of Gengou and Gay, obtained similar results. His experiments led him to conclude that our ideas of complement must be revised extensively, both Ehrlich's and Bordet's hypotheses failing to account for the conditions which he met with. Pfeiffer and Moreschi(30) and Friedberger and he(10) supposed that the disappearance of complement was in some way brought about by the union of precipitin and precipitable substance and varied with the amount of precipitation. They found that deflection occurs not only *in vitro* but also *in vivo*. They concluded that the phenomena formerly explained as depending upon the action of anti-complements and anti-amboceptors are actually due to complement deflection.

Sachs(32), replying in 1905 to Gay, worked over some experiments of Pfeiffer and Friedberger and concluded that the phenomenon of deflection is not due to precipitation, but is a function of the union of amboceptor with its corresponding anti-genetic substance. The deflection occurs even when no precipitate is formed and varies independently of the amount of precipitate, a result also obtained by Klein(17). Wassermann and Bruck(35) also point out that old bacterial extracts no longer precipitate, but still deflect and that the same holds true of tuberculin and anti-tuberculin. The Neisser-Wechsberg studies and one by Lipstein also supports Sachs's contention.

From the first, the phenomenon of deflection has been used in practical diagnosis. Bordet and Gengou elaborated a delicate test for the demonstration of anti-bodies specific for various bacteria. Subsequently, in 1902, Gengou(15) widened the application of this method, making it available for the determination of the presence of anti-bodies for the most diverse albuminoid solutions, such as milk, egg, etc. Neisser and Sachs(26, 27) employed the test for recognizing not the anti-body but the antigen, using their technique with special success in detecting minute traces of blood, and being able from these traces to determine the species from which the blood was derived. They advise the adoption of the method in forensic medicine, to supplement the precipitin test, and make mention of three cases in which they employed it. The advantages of the deflection method are: First, that it acts as a control for the precipitin method; second, that hæmolysis is a much more definite index than minute precipitation; third, that an opalescent serum is available for use; fourth, that it is not necessary to have such high potency serum as is needed in the precipitin test and that it is not necessary to wait for the clearing of the serum, which is so tedious in the older test.

During the course of 1905 and 1906, Wassermann and his co-workers have given a new application to this technique. They found that dissolved bacterial substances could be detected as readily as bacterial emulsion (*Med. Klin.* '05), and they were able to demonstrate minute traces of bacterial substance in blood derived from individuals suffering from various infections. By modifying the technique they were also able to recognize the early stages of reaction by proving the presence of minute traces of soluble anti-bacterial substances in diseased tissues. They then went one step further and made a novel application of this principle, applying it in the detection of minute traces of even unknown infectious agents in the infected body.

They perform the experiment in testing for anti-tuberculin and tuberculin in organs as follows:

A specific hæmolytic serum is first obtained and this is used with the corresponding anti-genetic blood corpuscles as an indicator of deflection of complement; for, if a specific serum and the corresponding red corpuscles are brought together and fresh serum is added, hæmolysis occurs if the fresh serum contains free complement, it does not occur if no free complement is present. Hæmolysis is therefore the index of whether or not free complement is present in the fresh serum. If a fresh serum which has been shown to contain complement loses its complementary action upon being treated with a mixture of tuberculin and anti-tuberculin, the complement is said to be deflected, provided suitable controls indicate that its disappearance is directly due to the union occurring between tuberculin and anti-tuberculin, and is not due to the tuberculin alone, to the anti-tuberculin alone, or to any other agent involved in the experiment.

It having been established that the union of tuberculin and anti-tuberculin deflects complement, any given tissue extract is tested for its content in tuberculin by noting whether the suspected extract and a stock preparation of anti-tuberculin will block the action of a serum known to contain active complement. On the other hand, a given tissue extract is tested for its content of anti-tuberculin by bringing together a stock preparation of tuberculin, the suspected extract and the complementary serum, and afterwards testing for hæmolysis.

The technique of the experiment is elaborate, a large number of controls are required and the test tubes must be scrupulously cleaned. The organs to be tested for tuberculin are removed under aseptic precautions and ground in a mortar with normal salt solution containing 0.5 per cent of carbolic acid. Five cubic centimeters of normal salt per 1 gram of organ are employed. The mixture is shaken in a machine for twenty-four hours at room temperature, centrifugalized clear of particles and the supernatant fluid drawn off and tested by mixing varying amounts with anti-tuberculin and fresh guinea pig serum. These three are brought together in a test tube and kept for one hour at 37° C. Finally, the mixture is added to test tubes containing 1 cubic centimeter of a 5 per cent suspension of serum-free sheep's corpuscles and twice the dissolving dose of inactivated rabbit's serum specifically hæmolytic for sheep's corpuscles. The volume of fluid in each tube is brought to 5 centimeters of salt solution. The test tubes are placed in the thermostat at 37° C. for two hours, are kept on ice over night, and in the morning are examined to determine the extent of hæmolysis which they may exhibit.

The technique in testing organ extracts for anti-tuberculin is the same, except that fixed quantities of a stock preparation of tuberculin and of complement are used with varying quantities of organ extract.

The following controls must accompany every test:

1. The organ extracts must be free from tissue particles, as these alone may bind complement.

2. The deflecting power of organ extracts alone and of tuberculin alone must be determined in the test for anti-tuberculin, and that of organ extract alone and of anti-tuberculin alone in the test for tuberculin. Occasionally, one of these substances exhibits a certain power of deflection.

3. The extract to be tested must be added to the sheep corpuscle-serum mixture to determine whether it alone effects hæmolysis.

4. A complete parallel series must be made at the same time, using nontuberculous organs from the species to which the test animal belongs.

5. The controls usual in every hæmolytic experiment must, of course, be set also—that is, sheep's corpuscles and salt solution; corpuscles and amboceptor; corpuscles and complement alone.

The hæmolytic dose of the specific rabbit's serum is determined in preliminary tests and likewise that of the complement serum. About twice the hæmolytic dose of amboceptor and the single dose of complement are employed in the experiment.[2]

Neisser and Sachs (28) state that deflection by serum and anti-serum fails if the anti-serum is first boiled, and they include a control with boiled anti-body in their forensic test. They also state that there is an optimum amount of the anti-serum causing deflection when added to complement and the suspended blood specimens. This amount is determined at a preliminary test in which varying amounts of anti-serum are employed with a fixed amount of complement, and a fixed amount (0.0001 cubic centimeter) of the test blood preparation. At first they recommended the employment of normal rabbit serum with sheep's corpuscles as the hæmolytic indicator, but later they advised the use of a specific serum with sheep's corpuscles.

The application of the deflection method by Wassermann and Bruck (35) to the study of tuberculosis apparently sheds light upon the bacterial and anti-bacterial reactions occurring in tuberculous individuals. In tuberculous organs and in the sera of animals treated with tuberculin, anti-tuberculin was demonstrated, while it was absent from the sera of thirteen patients at different stages of phthisis. They succeeded in demonstrating tuberculin also in the diseased organs. As the anti-tuberculin receptors are elaborated at the tuberculous focus, and as they usually remain sessile for a longer or shorter time, especially in human beings, it results that if a tuberculous patient receives an inoculation of tuberculin, nearly all of the substance becomes attached in the diseased focus, the tuberculin being concentrated from the plasma by the fixed anti-tuberculin, instead of being allowed to diffuse itself throughout the body fluids. The result of this concentration is that even a minute trace of tuberculin will produce the well-known tuberculin reaction.

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[2] For a clear description of the technique and of the controls necessary, see Wassermann and Bruck, *München. med. Wchnschr.* (1906), p. 2396, and Morgenroth and Stertz, *Virchow's Archiv.* (1907), **188**, p. 166.

A person inoculated with tuberculin will produce anti-tuberculin and eventually set it free, and possibly patients having had localized tuberculosis for a long time may also liberate anti-tuberculin into the circulation, although thirteen cases were negative. This circulating anti-tuberculin prevents the action of a subsequently inoculated dose of tuberculin. This fact accounts for the uncertainty of the tuberculin reaction in the more chronic cases of tuberculosis.

The amount of circulating tuberculin varies in individuals and in species. It was easiest to obtain an anti-tuberculin serum from cattle, next from guinea pigs, and most difficult to obtain it from human beings. Wassermann and Bruck claim that tuberculous cattle intended for importation into Germany are previously inoculated with tuberculin so that they will not react to the official tuberculin test.

Bruck(6) was able to demonstrate the tuberculin in the circulation five days after the onset of severe symptoms, during the course of acute general miliary tuberculosis, and again on the eighth day. On the twelfth the circulating tuberculin had disappeared and the serum contained anti-tuberculin, the patient's condition at the same time being improved. Finally, shortly before death, the anti-tuberculin disappeared and tuberculin was again demonstrable.

Bruck found the deflection test equally satisfactory in the diagnosis of pleural exudates, of the spinal fluid in meningococcus infection, of serum from typhoid fever patients, and in gonococcus infections(7). He states that Bockenheimer and Lexer could diagnose streptococcus sepsis and erysipelas by this method. A further study of meningococcus infection and immunity was made along these lines by Kolle and Wassermann(18). Müller and Oppenheim(21) also applied this test successfully in gonorrhoeal arthritis, Koch and Bruck in meningitis, and Eitner(9) in diagnosing leprosy.[3] Finally, Wassermann, A. Neisser, and Bruck(36) obtained a serum from monkeys immunized with syphilitic material and were able to obtain the specific deflection reaction with fresh extracts from syphilitic tissue, whereas the reaction was absent when fresh extracts of normal organs were employed. This result was

[3] Salomon, *Wien. med. Wchnsch.* (1907), **75**, 121, found the method of no value in the diagnosis of cancer.

confirmed later(25)—from 70 to 75.5 per cent of 163 cases giving a positive result either for syphilitic virus or for anti-syphilitic reaction product. They could demonstrate the virus in the blood of patients taking mercury. The anti-bodies were particularly abundant in the spinal fluid. Wassermann and Plaut(37) employed this last fact in the investigations of various paralyses. They found that the spinal fluid from a certain number of paralytics contained anti-syphilitic material which acted with fresh extracts of syphilitic organs to deflect complement. In some cases the spinal fluid of a paralytic had slightly greater deflecting action than his serum.[4] Wassermann(34) notes further that the test is available for the diagnosis of protozoön infections.

The results obtained by Wassermann and his colleagues with this method are certainly extremely interesting, in fact, almost startling, but the work in other laboratories has not yet given that confirmation to the claims of Wassermann which justifies us in accepting them unreservedly. The criticisms have to deal with the mechanism of the phenomenon on the one hand, and on the other, with its practical value in diagnosis. Furthermore, the application of this method in each particular infection raises a certain number of special questions.

The two principal hypotheses to explain the deflection have been outlined above. Weil and Nakayama, Axamit(1) and F. Mayer (quoted by Ranzi(31)) object to the test *in toto*, claiming that deflection can be produced by mixing a complement serum with bacterial extracts alone without any anti-bacterial serum; Weichardt(38) adds that deflection can be produced by electrolysis and by heat, and Uhlenhuth(33) found that disappearance of complement can be brought about by diverse substances producing mechanical precipitation, while Landsteiner and Stankovic(19) obtained similar results with colloidal suspensions. It has been observed that albuminous solutions in general absorb complement, in amounts proportional to the quantity of albumin. Wassermann and Plaut recently remarked upon the unaccountable and rapid alterations in deflecting power occurring in the extracts ready for use in the experiment and upon the differences between various extracts, suggesting that these differences and the sediment gradually forming in even the clearest extracts are autolytic in origin. They speak also of the need of determining that the total amount of albumin in the various mixtures is not sufficient to deflect complement, and they suggest that only extracts be used which in 0.1 cubic centimeter doses, cause no deflection.

Moreschi(24) has recently concluded that the test is not sufficiently delicate for the diagnosis of typhoid bacilli, as the bacilli alone are apt to cause deflection.[5]

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[4] Recently the value of this method in diagnosis of syphilis has been confirmed by A. Schutze, *Berl. Klin. Wchnsch.* (1907), 126, and by Marie and Levaditi, *Ann. de l'Inst. Pasteur* (1907), XXI, 138.

Furthermore, it has been claimed by Ganghofner and Langner(14) that deflection varies according to the amount and concentration of the ingredients and that an excess of hæmolytic amboceptor and of complement destroys the results. The same writers, although they find the reaction a very sensitive one, conclude that it is not adapted to the purposes of clinical diagnosis owing to the many uncertainties inherent in such a complicated procedure.

Gengou(16) tested the value of the phenomenon as a means of diagnosing tubercle bacillus, using a dozen different acid-fast bacilli for comparison. He concluded that there was nothing specific about the reaction, and found that serum from an animal immunized against one acid-fast bacillus gave the deflection test when employed with other species of acid-fast bacilli.

Neisser and Sachs(28), replying to Uhlenhuth's objections to their forensic test for human blood, emphasize clearly the necessity of numerous controls as a basis for judging the results obtained by this method and they show that Uhlenhuth neglected one control, namely, the testing of the deflecting power of the boiled specimen. They say that it remains for experience to demonstrate whether there are nonspecific deflections which will make the test valueless. The negative results of Uhlenhuth, and of Gengou are possibly due to incompleteness in their technical arrangements, especially to the incompleteness of the controls, and these results must be confirmed before they can be accepted.

If we summarize this work we may say that the general results form an addition to our knowledge of the mechanism of anti-body reaction, although the mechanism of deflection itself remains obscure. What relation this reaction bears to specific anti-complement action or to the absorption of complement by any albuminous serum, or to mechanical precipitation is not clear. Before pronouncing final judgment upon the results described in this review, we must await the work of others in this field, for the number of factors entering into each experiment increases the chances of error in technique and in judgment to an extent that can only be controlled by an abundance of evidence. This is particularly true of the class of experiments in which the serum is obtained from animals immunized by inoculation with infected organs, for here the amount of infecting material must be slight compared to that of organ material inoculated, and therefore there is great danger lest the organ-antibodies obscure the phenomenon due to any existing infection-antibodies. Of course any experimental evidence, either for or against, must be reënforced with all of the numerous controls demanded, or must prove the uselessness of these controls.

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[5] Leuchs, J.: *Berl. klin. Wchnsch.* (1907) 44, 68 and 107, has replied to Moreschi's criticism and contends that the deflection method is exceptionally accurate, delicate and specific in diagnosing typhoid fever from other bacteria such as paratyphoid.

It is quite possible that these studies will throw some light upon the nature of complement. The use of the method outlined above for ordinary clinical diagnosis is evidently out of the question at the present time because of the special training required, but if the results above described are confirmed, it may well furnish a means for aiding in the discovery of yet unknown infectious agents, and in diagnosis, when employed by suitably trained specialists.

Finally, we may refer once more to the interesting results obtained by applying this method to the study of the pathologic physiology of tuberculosis and of syphilis.

That the technique is a difficult and complicated one has been pointed out with sufficient emphasis. It remains to investigate critically the constancy and accuracy of the method and to remove or mitigate sources of error.

In concluding I wish to refer to one other suggestion made by Wassermann and Bruck(35) to the effect that the softening occurring in a tuberculous focus is brought about by the enzyme-like action of complement which is bound in the diseased tissues by the recurring union of tuberculin and anti-tuberculin. They think that the complement comes from both the broken down leucocytes and from the infiltrating small round cells. They attribute the fever partly to a nonspecific effect such as follows the injection of any bacterial preparation and partly to the specific action upon the tuberculous tissue of material derived from the bacilli. However, another possibility suggests itself. We know that complement is blocked in the union of tuberculin and anti-tuberculin, and on the other hand we must suppose that complement has some definite function to perform in the healthy body, probably some function having to do with metabolism. Now it seems reasonable to suppose that the removal of complement in normal function must upset the physiological processes occurring in the body. This may well be true even when the binding of complement occurs locally as in an isolated tuberculous focus, but in the reaction to a general infection, when bacterial substance and anti-substance are being bound in widely scattered parts of the body, or in the circulation, an enormous amount of complement may be blocked. It will be important to determine how much complement is so blocked during infections, whether definite complements are blocked by certain bacteria and whether there is any relation between the symptoms and the loss of complement.

It was recently found by Pfeiffer(30), that the loss of available complement in the living body interferes with bacteriolysis.

II. THE AGGRESSIN HYPOTHESIS.

Bail's studies in infection and immunity convinced him that bacteriolysis has little or nothing to do with true immunity against disease and that Ehrlich and his school have followed the wrong clue. He brings forward the following facts in support of this contention:

(*a*) A rabbit is susceptible to anthrax, although its serum is lytic for the germ, while the naturally immune fowl has a serum which is not lytic.[6]

(*b*) In rabbits immunized against anthrax, and in those passively immunized, there is no bacteriolytic power. Bacteria disappear gradually as the result of the phagocytic action of cells, chiefly marrow cells, but do not disappear suddenly because of lysis.

Sobernheim found that animals upon which he conferred a high immunity against anthrax exhibited no agglutinating action and no lytic action, whereas his guinea pigs, immunized with the anthrax bacilli which had been grown at high temperatures or killed, exhibited no true immunity, but a rich content in immune bodies, the richest anti-body serums, however, affording no true immunity. Bail found similar but not identical, relations to hold with typhoid.

(*c*) A comparison of the sera of sheep, rabbits and cattle shows great variations in the amount of immune bodies contained, while these animals are nearly equally susceptible to anthrax.

(*d*) In test-tube experiments, a bacteriolytic serum is blocked when the conditions are approximated to thōse in the body by adding body cells to the fluid.

(*e*) Evidence is furnished (Hoke) that what has been said of anthrax holds for other organisms.

(*f*) Bail concluded from his studies that animals which survive the Pfeiffer test owe their lives not to bacteriolysis, but to active phagocytosis. He considers that the virulence of bacteria depends not upon toxin production, but upon the power of the bacteria to multiply in the infected body. He shows that after intravenous inoculations of animals, one with anthrax, the other with hay bacillus, the early reactions are the same in the two animals, the difference appearing only when anthrax begins to multiply, and even very shortly before death the organs of the anthrax animal are sterile.

He then seeks to explain the fact that a very small number of pathogenic bacteria may manage to obtain a foothold in the infected body and survive the attacks of the agencies protecting their host. He assumes that the protective body-forces are interfered with in some way, at first locally and then generally, by certain obscure, hypothetical substances which he calls "aggressins."

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[6] For other examples of this condition see Deutsch and Feistmantel.

He states that these substances, which he occasionally refers to as simple manifestations of energy, are minute particles "of a special kind" thrown off or secreted by living, uninjured bacteria. These particles do not come from solution of the bacteria, nor are they extracts of bacteria; they are thrown off as the result of irritation, which, he claims, is a very different process. He emphasizes particularly and repeatedly the importance of having living and uninjured bacteria in order to obtain aggressins. The aggressins are capable of promoting infection; they may be *preformed* in the bacterium, but they are *active* only in the infected body. They are contained in the infectious exudates, such as serous exudates and inflammatory œdemas, and may be recovered by centrifugalization and sterilization at low temperature, being found then in the fluid part of the exudate. The substances are different from anything hitherto recognized, and their peculiarities are that they promote the development of an infection, and that they interfere with the action of the protective body-forces of the infected host, particularly, if not solely by inhibiting phagocytosis.

The properties of aggressin may be grouped together as follows:

1. Sterilized aggressin added to nonlethal doses of the corresponding bacillus makes these doses fatal. Thus sterilized typhoid aggressin added to a nonfatal dose of typhoid bacilli and inoculated, causes death. This action is apparently a stimulation of the bacteria so that they produce toxins. Apparently he does not distinguish between promotion of infection due to stimulation of the bacteria, and that due to inhibition of the protective mechanism of the host.
2. Fatal doses of bacteria act more severely and acutely upon the addition of aggressin.
3. Inoculations of aggressins into the peritoneal cavity suspend the action of a bacteriolytic serum introduced at the same time.
4. Inoculations of aggressin confer immunity which is entirely different from bactericidal immunity.
5. Heating for one-half hour to a temperature between 55° and 60° destroys aggressin, sterilization with chloroform, toluol, or dilute carbolic acid weakens it, and the centrifugated aggressin exudate is to be sterilized by heating to 44° in order to preserve it for future use.
6. Injections of aggressin alone are only slowly poisonous, and never acutely fatal.
7. A fatal dose produces a prolonged illness, with emaciation preceding death.
8. Not all exudates contain aggressin, and it varies considerably in quantity in different exudates, which otherwise appear identical.
9. The aggressin is usually most abundant in exudates which are rich in cells.
10. Aggressins vary according to their age and mode of production, and it is possible, by means of serial inoculations, to increase the aggressive action of successive exudates very considerably, although a point is finally reached at which the series may suddenly terminate.
11. Aggressins with bacteria block phagocytosis—that is, they are negatively chemiotactic—but aggressins alone act very slightly.

Basing his classification upon the power to produce aggressins, Bail divided bacteria into:

(*a*) True parasites which always produce aggressins; (*b*) half parasites, the aggressin power of which is very variable; (*c*) saprophytes.

The body may develop bactericidal immunity against the half parasites, but not against true parasites. As types of true parasites, he gives anthrax and chicken cholera, germs the aggressive action of which is unfailing. Artificial extracts of these germs have no aggressive action. Most epidemic diseases are produced by half parasites, as types of which he gives typhoid, cholera, dysentery, and plague. The toxicity of the half parasites is often extremely high.

The toxicity of a germ has nothing to do with aggressivity, and extremely toxic bacteria may be half parasites: the class depends upon the capacity of the germ "to infect under all conditions the susceptible living creature from its own natural habitat; that is, the animal that has succumbed." True parasites readily furnish an immunity by the aggressin method, but their extracts do not even confer a heightened resistance. Half parasites vary in their power to produce aggressins and to confer an anti-aggressive immunity.

There have been numerous objections to the claims made by Bail, and a series of articles has been published on this subject from different laboratories while Bail and his pupils have undertaken to support and strengthen the "aggressin doctrine" as Bail names his hypothesis. Pfeiffer and Friedberger(8) have shown that Bail's attempt to explain the blocking of the bacteriolytic action of a serum as due to aggressins is not tenable.

Wassermann and Citron(11) dispute Bail's claim that he is dealing with a new substance never before found outside of the infected body. They obtained aggressins (*a*) by growing bacteria in sterile exudates in test tubes; (*b*) by growing bacteria in sterile normal rabbit serum in test tubes, and (*c*) by making aqueous extracts of bacteria. They concluded that Bail had nothing new, but was dealing with dissolved bacterial substances. However, Citron(4) in a later article, notes that there are differences between the extracts prepared by different procedures, and believes that according to present evidence it is probable that the most effective aggressins can be extracted only from living bacteria.

Bouchard, as long ago as 1891, obtained typical aggressin actions by inoculating soluble bacterial products, together with the bacteria.

Strong(10) by using aqueous extracts of plague, obtained a mild immunity in monkeys which, however, was not demonstrable in guinea pigs. This is of particular importance from the fact that plague immunity is known to be non-bacteriolytic immunity, and Bail states repeatedly that non-bacteriolytic immunity is characteristic of aggressins

Doerr(5) also thinks that Bail is dealing with dissolved bacterial substances, as he obtained the precipitin reaction with aggressive exudates, and he brings forward criticism against the work of Bail, on the score of the inaccuracy of the so-called sublethal doses. He also found that aggressins are of themselves injurious, and that one aggressin promotes infection with a different species of bacillus.

Levy and Fornet(7) were able to demonstrate the aggressive nature of filtrates of 24- to 48-hour bouillon cultures of typhoid and paratyphoid bacilli. They found that these filtrates were "infection-promoting" to the extent of increasing fivefold the virulence of typhoid bacilli. The addition of filtrate or exudate aggressin to leucocytes blocked the phagocytic power against typhoid bacilli, which was otherwise present.[7]

Ballner(3) found that nonbacterial exudates obtained from guinea pigs increased the infectious action of Friedländer's bacillus in rabbits, in other words exercised an aggressive action. He could not obtain an anti-aggressin immunity with this germ.

Wolff-Eisner(13) reviews the aggressin literature at length and concludes that the aggressins are nothing but endotoxins from bacteria; that they alone are not present in sufficient amount to act strongly, but that, upon addition of fresh bacteria, a summation effect is obtained and the animal dies quickly. He thinks this also explains the negative chemiotaxis occurring with aggressins.

What part the aggressin plays in the blocking of leucocytosis is also in dispute. Citron, in studying meningococcus found that there was no relationship between aggressivity and power to block phagocytosis, while Salus' work raises the question as to whether the aggressin action is anti-chemiotactic at all, as the aggressin acts even though it is injected in a different part of the body from the bacteria.

On the other hand, Bail and his school have published a series of articles dealing with the aggressins in cholera, typhoid, dysentery, plague, tuberculosis, *Bacillus subtilis,* chicken cholera, hog cholera, pneumococcus and staphylococcus.

Bail has satisfied himself that aggressin action occurs in tuberculosis also, although in this case active phagocytosis continued after addition of aggressins. Koch long ago obtained the same action by the use of dead cultures, thus ruling out any vital action in the production of aggressins.

The last article from Bail is a lengthy rejoinder to the criticism of Wassermann and Citron. He contends that the extracts used by Wassermann and Citron are altogether different from aggressins, and that the various properties which they find in these extracts in no way alter the significance of the properties that he has attributed to aggressins. The points of difference between the "natural aggressin" obtained from infected animals by Bail's method, and the "artificial aggressins" obtained by the various extractive procedures are discussed at length.

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[7] Concerning Bail's statement that the aggressive property of body fluids is due to secretion products from bacteria, see v. Pirquet and Shick, "Die Serumkrankheit." Wien, 1905.

1. In the case of cholera, natural aggressins promote the infection, but do not destroy or stop lysis of the spirillum, nor cause loss of complement even when tested by the deflection test, the complement being present not only in the infected body but also in the aggressin exudate itself; whereas the artificial aggressin blocks lysis strongly and promotes infection by this means.

2. Similarly, when aggressin and a specific serum are inoculated into the peritoneal cavity of a guinea pig they do not block lysis, whereas the artificial aggressin with a specific serum does so.

3. Aqueous extracts of cholera and typhoid are so full of bacterial particles that they furnish a precipitate even in the presence of a normal serum; while natural aggressins may altogether fail to precipitate, even in the presence of a strong specific serum. The artificial aggressins do not occur in the course of natural infections, and hence are altogether removed from comparison with natural aggressins.

However, after disputing virulently the contention of Wassermann and Citron that Bail is dealing with extracts of bacterial substance, the latter makes certain concessions. He states that shaking bacteria in distilled water injures the bacteria. The extracts therefore do not fulfill the requirements of aggressins as defined by Bail, but he adds "although we acknowledge that some bacteria have loosely separated aggressin easily shaken off and to be obtained in small amounts." Again, "it is not impossible that aggressin preëxisting in the animal body may be recovered in small amounts *extra corpus;*" and he finally expresses a belief that some of Citron's artificial aggressins obtained from living bacteria contained small amounts of natural aggressin intermixed. Again, he states that not every exudate is aggressive, and not every aggressive exudate evidences pure aggressivity. In addition to the aggressive substances—if there are such—there may be present in an exudate or œdema "ordinary bacterial substances which are the original artificial aggressins of Wassermann and Citron and it is these latter which block hæmolysis."

He also states with regard to the solution of bacterial substances that in experiments with typhoid he almost always obtained aggressins in his exudates, but could prove by the method of complement deflection that ordinary bacterial particles were not in the exudate. The indications are that the aggressivity of exudates is in inverse ratio to the power to deflect complement. He holds that complement deflection is due to bacterial particles causing precipitation. No essential solution of bacterial substance, then, occurs in the animals, which fact agrees, he thinks, with the one that typhoid bacilli after inoculation rapidly acquire a resistance to bacteriolysis. However, with cholera, the case is different, the exudate containing dissolved bacterial substance in solution.

> "The conclusion is evident that it depends upon the course of an infection whether an animal has in its exudate larger or smaller amounts of dissolved bacterial substance."

The smaller the amount of dissolved bacterial substance in an exudate, as determined by complement deflection, the stronger is the aggressive action of the exudate.

These statements indicate a distinct recession from Bail's original position, for on the one hand he acknowledges that artificial bacterial extracts may contain aggressin and on the other, that aggressive exudates may contain in solution other bacterial substances together with aggressins.

Bail also discusses at length the relation between complement deflection and precipitation, particularly with regard to its bearing upon our understanding of aggressins. While artificial aggressins inhibit bacteriolysis, Bail notes that the third of a series of guinea pigs inoculated in turn with cholera, furnishes a natural aggressin which contains complement completing the action of anti-cholera serum. This complement action of the aggressin exudate is stronger in the third than in the second of a series of guinea pigs; and stronger in the second than in the first. This change is so regular that when it is absent it is safe to attribute it to an overdose of bacteria in setting the test.

In serial inoculations aggressivity increases until the danger arises that the series will cease from some cause not clearly understood. As the aggressivity increases, there is a decrease in the power of the exudate to block hæmolysis. Bail brings this forward in rebuttal of Citron's argument that the power to block hæmolysis furnishes an index of the aggressivity of the exudate. He discusses the phenomena of precipitation, complement deflection and multiplicity of complements. He agrees completely with Moreschi that deflection is not a function of the union of amboceptors with specific substances, but is due to gross or microscopic precipitation.

He states that he disregards the plurality of complement idea, which he thinks was overthrown by the discovery of complement deflection, and he agrees with Bordet in looking upon complement as a "single fermentative, complementative activity" of the body juices. However, he is not consistent in this position, for he finds that in some of his aggressin experiments the heart's blood of the animal contains hæmolytic complements; "and usually bactericidal complement not only for cholera and typhoid, but also for anthrax." This is a tacit confession that different complements are needed in these different reactions.

He does not agree with Citron in attributing to natural aggressin the power of complement absorption, but he gives examples showing how natural aggressins may lead to the blocking of hæmolysis.

Bail considers that the leucocytes are the chief source of complement. He thinks that extracts of bacilli hold back leucocytes more than natural aggressins do. He believes that inoculation of extracts acts just as does the inoculation of larger amounts of bacteria to promote the growth of the inoculated bacteria so that these, by means of their newly acquired aggressivity, restrain the growth of leucocytes. This is rather a complicated mode of procedure. Aggressin alone has no power to bind leucocytes, but only acts in the presence of bacteria. This he can not explain, but he found qualitative differences. The addition of cholera aggressin causes death by intoxication with no leucocytosis in a guinea pig previously inoculated with cholera and anti-cholera serum, the action being unlike that following the addition of cholera extracts or of sterile bouillon. This he brings forward as an additional proof of the difference between artificial and natural aggressins.

Turning to the question of immunity against infections, Bail holds that anti-bacterial immunity is not a true immunity, and that this can be given only by creating "anti-aggressin." These anti-aggressins are distinctly different from anti-bacterial substances, for the anti-aggressin immunity is certainly not bactericidal immunity in the cases of anthrax, chicken cholera, swine plague, and plague, dysentery, capsulated bacillus, and typhoid; while in the case of cholera he has never succeeded in obtaining an anti-aggressin immunity which did not also exhibit a bacteriolytic immunity. The determinations in these cases were made both by test-tube experiments and by Pfeiffer tests. The anti-aggressin immunity is a phagocytic immunity, at least in the case of typhoid.

However, even with strong anti-aggressive immunity, the infecting bacteria may survive and multiply in the host, but they cause no symptoms. Citron could also verify this, finding virulent hog cholera bacilli in an immunized animal after five and one-half months. He contends not only that bactericidal immunity is not a true immunity, but that this bactericidal power can not be deduced from the power of the serum to block hæmolysis; and, further, he claims that Wassermann and Citon are wrong in regarding the presence of amboceptors in an inoculated animal as an index that the reaction of immunity has occurred in those cases in which the cholera germ is still capable of living somewhere in the body of the immunized individual.

Bail gives the occurrence of strong phagocytosis in spite of severe infection as a characteristic of anti-aggressin immunity.

There is a negative stage after the inoculation of aggressin during which the animal exhibits marked hypersusceptibility to fresh inoculations of the germ. He also states that anti-aggressin immunity is one of the oldest methods of immunization, although its essentials were never before understood. He claims that the experiments of Wassermann, Ostertag and Citron with vaccination with suitably weakened bacilli, is nothing but a modified form of Pasteur's immunization, and adds: "We consider Pasteur immunity as the only true immunity, but it is nothing else than an aggressin immunity. This is shown by its not having a high content in anti-bodies."

A review of the work both for and against aggressins leaves it questionable whether Bail and his school have made any real contribution to bacteriology or immunity. It is by no means an easy matter, in many cases, to obtain a clear idea of their meaning, but some of the phenomena, which they describe as aggressive in nature, can hardly be due to any single cause and are probably to be explained by considering together a series of processes in the bacteria and another series in the infected host. Their claim that their aggressins are newly described substances has been nearly overthrown and most of the characteristics have been found under other conditions. As to the newness of anti-aggressin immunity, all doubt is set at rest by Bail's statement that it is the same as Pasteur's immunity.

The lessons to be learned from the aggressin work are not new; the same lessons are being taught by the workers upon opsonins and endotoxins, and they are:

1. That there are very great differences in activity between otherwise apparently identical strains of a species of bacterium; 2. That bacteria undergo some important alteration when transferred from a saprophytic to a parasitic existence; 3. That there are marked differences in the reactions occurring in various species of bacteria after they are inoculated into animals; 4. That the phenomena of agglutination, bacteriolysis or phagocytosis do not separately afford an explanation of anti-infectious immunity; 5. That the products of bacterial metabolism are numerous and differ among themselves, and that the products differ according to the pathogenicity of the bacterial strain.

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INFANT FEEDING AND ITS INFLUENCE UPON INFANT MORTALITY IN THE PHILIPPINE ISLANDS.[1]

[Copyrighted in the Philippine Islands, September, 1907.]

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I. INTRODUCTION.

Infant mortality is the most serious, important and urgent problem in preventive medicine in the Philippine Islands. The following figures of births and deaths for the *City of Manila* are taken from the records of the Bureau of Health:

Year.	Births.	Deaths.	Deaths (under 1 year of age).
1903	3,387	9,394	3,872
1904	6,341	11,357	6,029
1905	7,779	9,731	4,676
1906	8,679	9,486	4,218
Total	26,186	39,958	18,795

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[1] Read by abstract at the Fourth Annual Meeting of the Philippine Islands Medical Association, March 3, 1907.

The number 18,795, for the deaths of infants under 1 year of age, represents 47 per cent of the total mortality, and 71 per cent of the total recorded births.

No reliable figures are available from the provinces, but it is probable that the ratios will not be found materially to differ from the ones derived from the records in Manila, for whereas Manila has better sanitary conditions, and the services of hospitals, physicians and nurses are more regularly sought in the city, the provinces on the other hand have fresher and better milk and other foods, and infections are perhaps less frequent.

Three hundred and seven American children were born during the four years covered by these statistics, and while no accurate figures are available, the infant mortality among these was less than 5 per cent. Very similar results are obtained from statistics which one of us personally compiled, containing the record of 150 native children born to educated parents during the same period of time. However, in these two classes of patients rachitic tendencies, marasmus, anæmia and other damaging influences of malnutrition are sufficiently prevalent to prove that even in the absence of pure, fresh milk, we are not using the available foods to the best advantage.

On examining the recorded causes of death a little more closely we find some interesting and instructive figures regarding the death of 18,795 infants in Manila during the last four years. In 10,484 instances the cause of death is given as convulsions; in 1,416 (for three years only) diarrhœal diseases and dysentery; and in 780 (for three years only) simple meningitis is credited with the fatal result. In addition to these figures large numbers of certificates of death where the cause is given as tetanus, lack of care, debility, etc., are recorded. We but confirm the expressed opinion of Major E. C. Carter, formerly Commissioner of Health for the Philippine Islands, and many other prominent health officials and physicians, in stating that an overwhelming percentage of the 10,484 deaths reported as due to convulsions were in reality caused by gastro-intestinal diseases, the result of dietetic errors; this number may also safely be increased to include most of the cases of so-called tetanus, meningitis, debility, etc. Tetanus is prevalent in the Philippine Islands and it is to be expected in a certain number of babies because of the lack of care in treating the cord at birth, but in six patients which one of us was able to see during life, and in three more where Musgrave made post-mortem studies, the spasms were of other etiology and tetanus was not present.

Rickets and marasmus are not mentioned in the recorded causes of death, but both diseases are present among foreign and native children. We are convinced after a careful study of the records, the conditions and our own observations, that more than 75 per cent of the deaths of babies in Manila are primarily due to dietetic errors.

Major E. C. Carter, formerly Commissioner of Public Health, makes the following statement in his annual report for 1903:

> This excessive infant mortality is one common to all tropical countries. In Manila it appears chiefly to depend upon ignorance with respect to the proper care and feeding of young children and the difficulties of obtaining suitable food where nursing by the mother is for any reason impracticable or the supply of breast milk is insufficient. Fresh milk is almost impossible to obtain, and when obtained is usually of poor quality and contaminated by improper handling. In the absence of ice its preservation is practically an impossibility and no attempt is made to modify its constitution so as to conform more nearly in character to human breast milk. The destructive epidemics of rinderpest have also largely destroyed the few milch cattle formerly in the Islands and there has been but little resort to goats as a source of milk supply. The so-called Australian milk is costly, its use is not general and it is not well borne by many. The employment of prepared infant foods is understood by but few, and their cost places them beyond the reach of the poorer classes. The same applies to the use of condensed milk, which is at present the most available source of supply of food for infants. When used it is frequently improperly diluted or contaminated by the use of water from an impure source, giving rise to intestinal disorders and malnutrition which are rapidly fatal.

Major Carter in his annual report for 1904 again emphasized the statements given in his report for 1903 and in addition points to the invasion of the Islands by the "germ infected nursing bottle," he calls attention to the use of the milk of the coconut in feeding, and he started a campaign of education by distributing broadcast a bulletin on the care of children, which was prepared by a committee of native physicians.

Dr. V. G. Heiser, at present Director of the Bureau of Health, in his annual report for 1906 states that as a result of this bulletin the consumption of milk has increased by probably 500 per cent, nearly all the output being used as food for infants, and he further points out that notwithstanding this fact, there has been no appreciable decrease in infant mortality and he considers this to be due to the improper care and handling of both the milk and its containers. Dr. Heiser states that probably 95 per cent of the milk used is from the carabao.

However, even the large infant mortality, disastrous as it is, does not tell us of the pernicious influences brought about by the conditions which produce this mortality, upon those who escape death in infancy. A large percentage additional to the 71 per cent of infants who die before the end of their first year, is to be credited to those who are left with crippled constitutions and who later become unnecessarily susceptible to other diseases. This supplementary mortality must be charged to the same lack of care of the helpless ones, which makes the loss of so many infants possible.

The problem of infant mortality and infant feeding is, of course, a fundamental one in all parts of the world and it is particularly so in cities, but here it is even more complex than it is in other places because of at least two important reasons—one, the almost complete absence of good, fresh milk, and the other, the lack of satisfactory literature bearing upon the subject. So far as we are aware, there is not a book or exhaustive article which deals with the problem of infant feeding in countries where a supply of fresh milk is lacking and where, in addition, the ability of mothers to nurse their offspring is reduced to a minimum for reasons which we will discuss below.

II. HABITS AND CUSTOMS OF THE PEOPLE.

A. THE NATIVE MOTHER.

The habits and customs of the people deserve a passing notice if we consider that the feeding of the child begins with conception. A parturient native woman is generally a happy one, proud of her condition, and she as a rule continues her regular habits and vocation during the period of her gestation. The diet during this time is of the usual variety, which contains an excess of carbohydrates.

Confinement, particularly among the poorer class of people, is of a very primitive type. In Manila, over 50 per cent of the children are born without medical attendance, usually with the aid of a midwife whose presence often does more harm than good. Mechanical assistance is afforded to the mother by passing a rope or folded sheet around the body just above the fundus, whereupon one or more friends pull upon the ends. The bad results to both mother and child following this and many other even more dangerous procedures, need no discussion here.

B. THE NATIVE CHILD.

The native child usually has the best attention which its mother can afford and which she knows how to administer, but the density of the ignorance and even of the superstition which exists among the uneducated classes is remarkable, and in addition, generations of self-medication have resulted in the extensive adoption of customs which are of the most pernicious type, often maintained with a persistence which seems culpable and vicious in the face of gratuitous enlightenment.

Breast feeding is probably attempted in almost every case, but the percentage of exclusively breast-fed children is certainly much smaller than it is in many other countries. This is due to several causes among which may be mentioned infections, injuries to the mother at childbirth and, most important of all, the lack of sufficient and proper food for the mother during the puerperum; we also must remember that the hereditary influences of generations of artificial feeding are always present to contend with. The average Filipino woman is poorly developed, and even those who have borne several children usually have small breasts, so that the milk-giving capacity is at a minimum. The conditions which have been outlined and many others, bring about the necessity of instituting artificial food for breast milk in infant feeding to an extent, and at an age of the infant, probably not surpassed if it is equaled in any other country.

Mixed feeding is usually the first departure from exclusive breast feeding; it is instituted early and continued as long as milk may be obtained from the breast, or until long after the child should be weaned. The only contraindication to breast feeding among the poorer classes is the lack of milk; no cause for discontinuation being found in tuberculosis, in other infectious diseases or in pregnancy. The accessory articles of diet in mixed feeding are very numerous, and the methods of preparation and administration primitive, but as they are identical with the ones administered in pure artificial feeding, their consideration will be postponed until that heading is reached.

Artificial feeding is rarely resorted to entirely so long as the breast milk continues, but in most of the instances of mixed feeding the nourishment which is taken is mainly from artificial sources, which will now be discussed in detail.

Milk is the most important of the articles of diet. That of cows, goats, and carabaos is used and a large assortment of canned and condensed milks is also employed. Other prepared foods of foreign manufacture are given to a limited extend in addition to the different milks, and homemade preparations in large variety make up the bulk of the infant food among the poor people. Most of the latter articles are mixtures of starch and sugar, prepared without proper regard to cleanliness, and among these may be mentioned the rice sticks, made by boiling rice and sugar until a glue is formed, which can be molded into a stick to be sucked by the child. Potatoes, bananas and other fruits are given at a very early age, and meat feeding is very frequently instituted before the eruption of the temporary teeth. It is not an infrequent occurrence at autopsies to find pieces of undigested meat in the stomachs of four months' old children. Not only does the quality of the foods and their indiscriminate use, regardless of the age of the child, bring disaster, but the character and percentage of the diluents also contribute very much to the bad results. Throughout the sad story—and this is the pity of it—nothing but the ignorance of the people is to blame for the results.

C. THE FOREIGN MOTHER AND CHILD.

In Manila the social customs are such that in general the foreign woman neither cares so well for herself nor does she make as good a mother as she would in her home country. Nevertheless, the children of foreigners and of the educated class of Filipinos thrive, although surrounded by the same climatic and other unavoidable evils which encompass those of the ignorant classes. The infant mortality among foreigners and wealthy natives compares favorably with that in other lands; the children are fairly strong and not overburdened with the diseases of malnutrition so common in all countries.

III. A STUDY OF THE CONDITIONS.

A very complex condition appears before us for study, to judge from the preceding brief and incomplete résumé of facts. However, the limits of this paper will allow of the consideration of but one of the problems, namely, that of infant feeding, and in discussing this topic we will first take up a consideration of the available materials.

THE AVAILABLE FOOD SUPPLY.

Milk is the first of the articles to be considered and it may be classified as either fresh or preserved. Among the fresh milks we will consider human, cows', carabaos' and goats' milk and among the preserved varieties we have many brands of the sterilized, condensed and malted type.

(*a*) HUMAN MILK.

The percentage of American mothers who are able to nurse their children in Manila is higher than it is in general in American cities. The quality of the milk is of a very good average, perhaps with a tendency to a high sugar index, with a correspondingly lowered proteid and fat content, as is shown in the following table:

TABLE No. 1.—*Analyses of ten samples of breast milk from American women who have infants from 20 days to 2 months old.*

No.	Specific gravity.	Water.	Solids.	Ash.	Fat.	Sugar.	Proteid.
1	1.032	88.80	11.20	0.20	2.40	6.10	2.50
2	1.030	87.19	12.81	.11	5.55	6.07	1.08
3	1.029	88.74	11.26	.33	3.48	6.31	1.14
4	1.030	86.80	13.20	.14	4.44	7.58	1.04
5	1.027	84.47	15.53	.11	7.27	7.16	.99
6	1.027	84.44	15.56	.25	6.36	6.98	1.97
7	1.033	89.45	10.55	.26	1.90	6.81	1.58
8	1.030	87.22	12.78	.27	5.48	6.23	1.42
9	1.029	88.62	11.38	.34	3.61	6.28	1.18
10	1.032	89.41	10.59	.23	1.92	6.78	1.60
Average	1.0299	87.46	12.48	.22	4.24	6.63	1.45

The analyses of breast milk among the better class of Filipino women who can and do partake of a liberal diet and who otherwise obey the mandates of hygiene also gives a good average, as shown in Table No. 2.

TABLE NO. 2.—*Analyses of breast milk taken from the wealthier class of Filipino women.*

No.	Specific gravity.	Water.	Solids.	Ash.	Fat.	Sugar.	Proteid.
1	1.030	86.22	13.78	0.27	5.78	6.33	1.40
2	1.031	86.78	13.22	.17	4.34	7.76	.95
3	1.027	84.43	15.57	.26	6.34	6.98	1.97
4	1.029	85.49	14.51	.11	6.64	6.07	1.69
5	1.028	88.17	11.83	.41	3.35	5.91	2.16
6	1.030	88.30	11.70	.23	3.21	7.18	1.08
7	1.031	88.82	11.18	.12	2.61	7.59	.92
Average	1.029	86.88	13.11	.22	5.38	6.83	1.45

However, a study of the breast milk among the poor people gives some interesting figures. In the first place, the quantity is small and the period of active lactation short. Analyses of ten samples of milk show a persistent high specific gravity, high sugar index, with low proteid and fat. This is apparent from the following table:

TABLE NO. 3.—*Analyses of ten specimens of breast milk from native women of the poorer classes, with infants from 10 days to 1 month old.*

No.	Specific gravity.	Water.	Solids.	Ash.	Fat.	Sugar.	Proteid.
1	1.035	86.83	13.17	0.11	2.84	8.70	1.52
2	1.038	87.29	12.71	.34	2.55	8.29	1.53
3	1.034	86.91	13.09	.15	2.33	9.20	1.41
4	1.036	87.43	12.57	.13	2.98	8.08	1.38
5	1.030	87.20	12.80	.11	3.58	7.11	1.40
6	1.030	86.60	13.40	.14	4.50	7.62	1.14
7	1.030	86.71	13.29	.15	4.02	7.98	1.14
8	1.030	88.04	11.96	.09	2.67	8.14	1.02
9	1.030	86.96	13.04	.16	3.97	7.83	1.08
10	1.031	87.97	12.03	.15	1.31	6.87	1.31
Average	1.033	87.19	12.81	.15	3.07	7.98	1.29

A study of these analyses shows that even in breast feeding in Manila there are unusual problems to be met. In order fully to appreciate this fact we have taken the averages from the preceding tables and placed them in the following one, which also gives average human milk analyses from two representative sources, for comparative purposes.

TABLE No. 4.—*Showing comparison between human milk analyzed by several authors and the findings in Manila.*

Source.	Fat.	Sugar.	Total proteid.	Casein.	Whey proteid.
Averages given by Rotch	3.00–4.00	6.00–7.00	1.00–2.00	0.59	1.23
Averages given by König	3.80	6.20	2.30	1.00	1.30
Average from American women in Manila	4.24	6.63	1.45		
Average from Filipino women in Manila of—					
Better class	5.38	6.83	1.45		
Poor class	3.07	7.98	1.29		

Some of the discrepancies between the analyses of human milks from women of the Tropics and of those given for other countries are usually explained by the difference in diet, and this also partially makes clear the variations due to the racial differences in women living in the Philippine Islands. However, there are other influences such as heredity, nervous temperament, climate, etc., which here play an active part and which must not only be reckoned with in determining what is abnormal in the mother's milk, but also in *fixing standards of normality* for the infant's requirements with reference to nutrition. For example, according to our standards the high sugar index and the low fat content found in the breast milk of the native women of the lower classes are too far from the normal to accord with our present conception of the physiology of nutrition of the infant and, according to the same standards, they are partly responsible for the gastro-intestinal disturbances and for some of the malnutrition and lack of development of the children of these classes.

However, it is not at all certain but that our standards of averages may need some adjustment in this respect, and it may be true that the child of parentage which for generations has lived in the Tropics may demand more sugar and less fat than we have been accustomed to recognize as normal.

(*b*) OTHER FRESH MILKS.

1. *Fresh cows' milk,* because of the dearth of horned animals, due to rinderpest, is very difficult to obtain in any considerable quantity and its price is, and for some years it must remain, prohibitive for most of the people. Adulteration of the available cows' milk is exceedingly common and flagrant; the adulterants consist of carabaos' or goats' milk, canned milks, and often of chalk, limewater and other substances. Bacteriological examinations show this milk to be unfit for use. After repeated efforts we were unable to obtain ten samples from the market in order to submit a table of comparative results.

Examinations of ten samples of fresh cows' milk from the Bureau of Agriculture in Manila are given below. These animals are well selected and are specially cared for by Government officials.

TABLE NO. 5.—*Analyses of ten samples of cows' milk from stall-fed animals, the property of the Bureau of Agriculture in Manila.*

No.	Specific gravity.	Water.	Solids.	Ash.	Fat.	Sugar.	Proteid.
1	1.034	89.78	10.22	0.72	3.73	2.39	3.38
2	1.032	89.90	10.10	.63	2.63	4.36	2.48
3	1.035	88.22	11.78	.65	3.44	4.57	3.12
4	1.033	89.50	10.50	.60	2.09	5.07	2.74
5	1.031	89.55	10.45	.75	2.52	3.87	3.31
6	1.032	89.22	10.78	.71	2.42	4.40	3.25
7	1.033	90.59	9.41	.65	1.64	4.00	3.12
8	1.028	87.97	12.03	.65	4.34	4.11	2.93
9	1.034	86.38	13.62	.70	3.73	4.86	4.33
10	1.032	89.80	10.20	.60	1.66	5.07	2.87
Average	1.032	89.09	10.91	.66	2.82	4.21	3.15

In the following table the averages from the preceding ones are compared with those given for fresh cows' milk by two distinguished authors:

TABLE NO. 6.—*Showing comparison between the results of examinations of cows' milk as given by other authors and as determined in Manila.*

	Fat.	Sugar.	Total proteid.	Casein.	Lact-albumin.
Average fresh cows' milk (König)	3.70	4.90	3.50	3.00	0.50
Average fresh cows' milk (Rotch)	4.00	4.75	3.50	2.66	.84
Average fresh cows' milk Manila	2.82	4.21	3.15		

The contents of fresh cows' milk varies between very wide limits in all parts of the world, but in Manila this variation is excessive and is in part due to the alteration in the kind of food to which the cows have been accustomed, to the difference in the care of the animals and to difficulty of securing *average* milks because of the lack of large herds of cattle. As it is impossible to obtain an average, it is necessary to examine the milk from each animal or herd before it can intelligently be modified for infants' use, and even after these precautions have been taken many of these milks are of a composition which renders proper modification very difficult. The worst cases of rickets to be seen in the Philippine Islands are often observed among children who are fed upon so-called fresh, cows' milk. The average bacterial count in five samples of milk bought in the market was 2,160,000 and the average from five samples from cows from private families gave 765,000. As a result of personal observation regarding the care given to milk, animals, vessels and the surroundings of the stables in general, we can not recommend the use of unboiled, fresh milk in Manila obtainable in the open market for human food, and until conditions are much improved, its use as food for infants seems almost criminal.

2. *Goats' milk* is to some extent employed in Manila as an infant food, but the supply has been limited, and it is very dirty and of poor quality as it is put on the market. The quality is shown by the following table:

TABLE NO. 7.—*Showing the results of the examination of eight samples of ordinary goats' milk in Manila.*

No.	Specific gravity.	Water.	Solids.	Ash.	Fat.	Sugar.	Proteid.
1	1.032	85.69	14.31	1.97	5.18	3.66	3.50
2	1.030	84.11	15.89	1.82	6.56	4.13	3.38
3	1.035	84.76	15.24	2.07	4.95	4.01	4.21
4	1.036	86.66	13.34	2.07	3.04	3.77	4.46
5	1.034	82.39	17.61	2.45	7.73	2.39	5.04
6	1.034	85.48	14.52	2.00	5.01	3.81	3.70
7	1.035	80.20	19.80	1.75	8.95	5.53	3.57
8	1.030	88.03	11.97	2.02	4.41	1.02	4.52
Average	1.033	84.66	15.33	2.02	5.73	3.54	4.05

The Government has recently imported some milch goats from Malta and if suitable food is available and conditions are otherwise found to be satisfactory for the propagation of these animals, this importation will result in doing much good. The reaction of the changed environment upon these animals is still problematical and a study of the milk to be obtained from them after acclimatization remains to be made. Analyses of seven samples of milk from this herd after the animals had been two months in Manila are given in the following table:

TABLE NO. 8.—*Showing the ressult of the examination of seven samples of goats' milk from a herd of Maltese goats imported by the Government.*

No.	Specific gravity.	Water.	Solids	Ash.	Fat.	Sugar.	Proteid.
1	1.028	87.97	12.03	0.63	4.78	3.31	3.31
2	1.029	87.39	12.61	.67	4.29	4.34	3.31
3	1.031	86.96	13.04	.59	4.83	4.34	3.38
4	1.030	87.57	12.44	.79	4.57	3.45	3.63
5	1.032	87.97	12.03	.63	3.24	5.87	2.29
6	1.030	84.90	15.10	.60	6.66	4.40	3.44
7	1.030	86.97	12.03	.59	4.88	4.31	3.25
Average	1.029	87.10	12.90	.64	4.73	4.27	3.23

Averages from the two preceding tables, compared with those of goats' milk given by one American and one European author, are placed in the following table:

TABLE NO. 9.—*Showing comparison between results of the examination of goats' milk as given by other authors and as determined in Manila.*

Kind of animal.	Fat.	Sugar.	Total proteids.	Casein.	Lact-albumin.
American goats (Rotch)	4.30	4.00	4.70	----------	----------
European goats (König)	4.80	4.40	4.30	3.20	1.10
Maltese goats in Manila	4.75	4.27	3.23	----------	----------
Native goats in Manila	5.73	3,54	4.05	----------	----------

The same objections as those already mentioned for cows' milk must obtain until the general conditions for securing and caring for this milk have been much improved. All of these milks as they appear on the open market are exceedingly dangerous bacteriologically, and the lack of large herds makes it impossible to obtain any quantity of *average, mixed milk.*

3. *Carabaos' milk* is extensively used as an infant food among the poorer people and it is the least adapted to this purpose of any which we have as yet studied. The results of analyses of 6 samples of carabaos' milk made by Charles L. Bliss, formerly of the Chemical Laboratory, Bureau of Science, are given in the following table:

TABLE NO. 10.—*Showing results of analyses of carabao milks.*

	1.	2.	3.	4.	5.	6.	Averages.
Age of animal	15 yrs.	13 yrs.	6 yrs.	8 yrs.	16 yrs.	10 yrs.	----------
Age of calf	3 mos.	2 mos.	2 yrs.	1 yr.	4 mos.	7 mos.	----------
Amount of milk	1,700	1,550	----------	----------	1,000	1,500	----------
Specific gravity	1.040	1.037	1.032	1.032	1.038	1.039	1.036+
Fat	12.76	9.55	13.22	11.56	8.65	8.36	10.63
Sugar	3.62	3.82	3.34	2.90	4.19	4.50	3.73
Proteid	6.97	6.75	6.78	6.34	5.54	5.46	6.31
Ash	.99	.09	.90	.82	.85	.85	.88
Total	24.34	20.02	24.24	21.62	19.23	19.17	21.44
Solids by evaporation	25.28	20.83	24.24	22.52	19.92	19.79	22.09
Water	74.72	79.17	75.76	77.48	80.08	80.21	77.90
Solids not fat	12.52	11.28	11.02	10.96	11.27	11.43	11.41

Two samples, 3 and 4, were treated with dilute hydrochloric acid. The coagulum found was compared with that produced in fresh cows' milk similarly treated. To 10 cubic centimeters of each of these milks 5 drops of hydrochloric acid of 1.12 specific gravity were added and the whole heated to 37–38° C. Both carabao milks became solid immediately. The coagulum very closely resembled that produced by rennet in cows' milk. The result with the cows' milk was negative. This would indicate that carabao milk would need to be diluted considerably for infants' feeding, as it would coagulate.

Carabaos' milk as it is obtained and marketed in Manila, is about as dangerous and dirty a mixture as it is possible to have. Bacteriologically, it is excessively contaminated and chemically it is not easy properly to modify for the use of infants.

(c) PRESERVED MILKS.

A great variety of these are imported into Manila, and in enormous quantities. The records of the Bureau of Customs show that 4,041,703 pounds of condensed milk, with an approximate value of ₱800,000 and 59,809 gallons of fresh milk, with an approximate value of ₱147,000 were imported into Manila during the year 1906.

The following Table No. 11 shows the principal brands on the market, with the results of the analyses of samples, each obtained in the open market:

TABLE No. 11.—*Showing results of analyses of samples obtained in the open market of some of the most generally used preserved milks in Manila.*[a]

Name.	Specific gravity.	Water.	Solids.	Ash.	Fat.	Sugar.	Proteid.	Remarks.
Bear milk	1.029	87.87	12.13	0.67	4.27	4.06	3.07	Average from 3 samples. Sterile. No concentration; no adulterants.
Dragon milk	1.029	87.66	12.33	.76	4.12	4.35	3.11	Do.
Weribest milk	1.032	88.20	11.20	.71	3.24	4.75	3.10	Sterile; no concentration; no adulterants.
Highland evaporated cream.	[b]1.077	70.61	29.38	1.80	7.86	11.13	8.40	Average of 3 samples. Sterile; concentrated; no adulterants.
Bear concentrated.	[b]1.040	70.95	29.05	1.75	9.67	9.79	7.84	Sterile; concentrated; no adulterants.
Bear cream[c]	.943	55.95	44.09	.46	38.07	3.22	2.29	Average from 2 samples. Sterile; slightly concentrated; no adulterants.
Australian	[b]1.045	60.85	39.15	2.32	14.00	13.52	9.31	Cold storage. Concentrated. Contained a compound of boron. 3 samples.
Sego evaporated cream.	[b]1.033	73.25	26.75	1.45	10.08	8.29	6.95	Sterile; concentrated; no adulterants.
Tulip evaporated cream.	[b]1.034	71.18	28.82	1.57	10.91	9.20	7.14	Do.
St. Charles evaporated cream.	[b]1.076	70.58	29.42	1.88	7.96	11.34	8.21	Do.
Pet evaporated cream.	[b]1.034	72.00	28.00	1.20	9.75	9.91	7.14	Do.
Cows' head evaporated cream.	[b]1.04	67.58	32.42	1.80	10.33	12.19	8.10	No preservatives. Concentrated; sterile.
Ideal evaporated cream.	-------	68.27	-------	1.85	10.10	11.03	7.36	Sterile; concentrated; no adulterants.

[a] Since this table was prepared a number of other milks have been received and their analyses will be found in Table No. 14.

[b] Diluted one-half.

[c] One sample of this cream contained 9.06 per cent of sugar and 20.85 per cent fat.

TABLE NO. 12.—*Showing result of examination of samples obtained in the open market of some of the most generally used sweetened, condensed milks.*

Name of condensed milk.	Total solids.	Water.	Ash.	Fat.	Milk sugar.	Pro-teid.	Cane sugar.	Remarks.
Nestles	25.25	--------	1.84	10.62	12.53	7.90	40.56	Analyzed by Chapin.[a]
Eagle	28.41	--------	1.80	8.44	11.69	7.23	41.52	Do.
Milkmaid[b]	27.43	72.57	1.73	9.63	11.00	4.33	45.88	Milk solids, 26.69.
Perfection	26.86	73.14	1.94	.50	12.06	8.54	50.10	Milk solids, 23.04. A condensed and sweetened skimmed milk.
Meadow brand	23.63	74.37	1.80	4.00	11.55	8.70	48.32	Milk solids, 26.05.
Gold seal	15.23	84.77	1.75	8.25	9.75	7.46	56.57	Milk solids, 27.21.

[a] Chapin: Theory and Practice of Infant Feeding. Second edition.

[b] This milk is popularly known as the "Señorita brand."

The milks which have been tabulated above fall into three principal classes; one of partially concentrated milks, sterilized, bottled, shipped and kept in cold storage, another of pure cows' milk canned without any other alteration than simple sterilization by heat, and the last, of the various types of condensed milks, including the so-called evaporated creams.

1. *Cold storage milk.*—The only brand of this milk on the market is the "Australian" which, as the name implies, is brought from Australia and the analyses of samples are given in Table No. 11. It is a concentrated milk and the three samples analyzed contained a compound of boron.

2. *Sterilized, uncondensed milks* are sold in Manila in five brands, and analyses of samples of all are given in Tables Nos. 11 and 15 and the accompanying footnote.[2] These are all free from adulterants and preservatives and, as may be noted from the table, they are without exception excellent, mixed milks. To judge from the homogeneous distribution of the fat and from other considerations it is probable that at least a portion of each of them consists of goat's milk. The proteid constituents of this type have not as yet been carefully examined, but preliminary experiments make it appear probable that the caseïnogen radical is considerably in excess of the normal amount in human milks, as is true in all other milks from the lower animals. However this may be, they are, when properly modified upon a percentage basis, very satisfactory as infant foods, giving as good results as may be obtained with any *sterilized* food. If one of the three brands of pure canned cream on the market

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[2] Since this article was finished two new milk products have appeared upon the Manila market, namely, "Natura" and "Butterfly" brands. Analyses of samples of these two brands are as follows:

Name.	Specific gravity.	Water.	Solids.	Ash.	Fat.	Sugar.	Proteid.
Natura milk	1.030	87.00	13.00	0.69	3.22	6.22	2.87
Natura cream	----------	65.57	34.43	.54	27.13	4.34	2.42
Butterfly milk	----------	87.24	12.76	.73	2.92	6.95	2.16
Butterfly cream	----------	64.92	35.08	.62	26.95	4.00	3.51

is used with them, any reasonable percentage formula may be obtained, as will be shown in chapter four, and by the use of such combinations many small patients in Manila who are difficult to feed may be materially benefited.

3. Condensed whole milk.—There is a considerable variety of this class of preserved milks on the market (see Tables Nos. 11 and 15); many are good and some are made from a poor quality of milk, and in a few instances substances have been added which may be deleterious to the health of the consumer. These brands are very extensively advertised and used throughout the Orient for cooking and other household purposes and as a food for infants. They may be so modified for the latter purpose by the percentage method as to make very good substitutes for fresh milk, but when they are simply diluted seven to fourteen times with water, a procedure often recommended and very generally practiced here, they are much too low in fat for the proper nourishment of the infant.[3]

4. Condensed milk, sweetened or otherwise modified.—Analyses some of which are quoted and others made in this laboratory of samples of the principal brands of this class on the Manila market are given in Tables Nos. 12 and 15. Many are honest products, and when they are diluted and modified on a percentage basis, they may be used in the absence of more satisfactory food. These milks all contain large percentages of cane sugar and from some of them a portion of the natural cream has been removed before the process of condensation was begun.

5. Malted milks.—There are two varieties on the market, and copies of analyses of samples of both are given in Table No. 13. These foods are extensively employed in the Philippine Islands and with proper modifications, they can be altered so as to be used as infant foods in many instances. The greatest objection to them lies in the large amount of insoluble carbohydrates which they contain.

6. Cream.—There are three brands of cream sterilized in tins on the market and analyses of samples are given in Table No. 12 and footnote page 373. They are most useful articles in preparing percentage foods from preserved milks, because of the high fat percentage (26+ to 38+ per cent) and the low proteid content in two of them.

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[3] Many of the so-called brands of evaporated creams are merely unsweetened condensed milks, having something of the consistency and appearance, but not the taste or physical characteristics of cream. It is asserted by some that concentration to give a sufficient amount of fat warrants the term "cream" as distinct from milk, and that it really makes the product cream, and not condensed milk; but cream does not contain abnormal amounts of casein, lactose and mineral matters, for it is nothing more or less than milk containing an excess of butter fat. The "evaporated creams" contain as many times the normal percentages of all the constituents of milk as there are volumes of milk condensed. It is true that in each case a statement appears on the label that the "cream" is an unsweetened condensed milk, but this information is generally given in such a way as not to attract attention.

(*d*) FOODS OTHER THAN MILK.

We have homemade and imported varieties and diluents of this class.

1. The homemade foods of the Philippine Islands are considerable in number; for the greater part they consist of starch and sugar mixtures. Some of these, which are more or less peculiar to the country, may be mentioned. The rice stick which was referred to above, if it were carefully made and not fed to children too early in life would compare favorably in value with some of the other starch and sugar preparations which sell for high prices.

Coconut milk is used in the Philippines to a limited extent as a food for infants. No literature on the subject is to be found here, but the habit of using it is certainly peculiar to people of the Tropics. The composition of this substance varies much with the age of the coconut from which it comes. Walker[4] notes some of its peculiarities and gives a few analyses, which however were not made with a view of determining its nutritional value as an infant food. This question should be carefully investigated, for coconuts are everywhere present in the Philippine Islands and it is very probable that a valuable enzymic diluent or even food may be found in them. The country contains many fruits which should contain substances much needed in those food mixtures which are sterilized, and which therefore are free from enzymes.

2. Imported foods other than milk are not as yet used to the same extent as they are in many other countries, but there are several varieties on the market, some of these are good in the sense that they are honestly made. Analyses *not made in this Laboratory* of a few which are used in greatest quantity in Manila will be found in the following table:

TABLE NO. 13.—*Showing analyses of imported foods other than milk.*

Name.	Analyst.	Moisture.	Fat.	Proteids.	Soluble carbohydrates.	Insoluble carbohydrates.	Ash.
Allenbury's food No. 1	Chapin	5.70	14.00	9.70	66.85	----------	3.75
Allenbury's food No. 2	do	3.90	12.30	9.20	72.10	----------	3.50
Horlicks malted milk	do	2.55	1.41	14.00	63.87	15.68	3.57
Nestles milk food	do	2.18	4.45	10.72	43.84	[a]35.34	1.60
Mellins food	do	4.72	.30	10.10	82.06	----------	3.50
Carmicks soluble food	do	5.69	2.18	16.60	38.21	38.54	2.78
Imperial granum	do	6.04	.72	13.77	3.94	67.46	.49
Ridges food	do	8.12	.48	13.83	5.02	69.24	.53
Health Food Companies barley.	do	10.92	.89	6.98	----------	80.35	.86
Robinson's patent barley	do	9.41	.41	7.46	2.91	78.66	.94
Bordens' malted milk	Holt	3.25	5.41	14.04	74.31		2.99

[a] Starch.

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[4] *This Journal* (1906) 1, 58.

3. *Diluents* are most important aids in infant feeding and they deserve especial notice here, because of the necessity for care as to the quality of water and of the other substances used. The following Table No. 14 gives the contents of a few of the principal diluents used in preparing foods for infants.

TABLE NO. 14.—*Giving analyses of a few diluents for baby food* (*taken from Rotch*).

Substance.	Preparation.	Solids.	Ash.	Fat.	Total Proteid.	Casein.	Lactalbumin.	Sugar.	Starch
Oat meal water	2 oz. cooked ½ hour, finished product 1,000 cc.	3.52	0.03	0.08	0.41	------	------	------	2.99
Do	3 oz. cooked ½ hour, finished product 1,000 cc.	5.21	.05	.11	.47	------	------	------	4.58
Barley meal water.	2 oz. cooked ½ hour, finished product 1,000 cc.	3.48	.03	.02	.54	------	------	------	2.88
Do	3 oz. cooked ½ hour, finished product 1,000 cc.	5.19	.04	.03	.68	------	------	------	4.43
Fat free cows' milk.	------	------	------	.05	3.55	------	------	5.00	------
Whey from cows' milk.	------	------	------	------	------	------	0.90	5.10	------

The contents of these diluents is not generally considered in percentage feeding, but in feeding infants in the Tropics, where the percentages are not as accurate as they are at home, it appears more advisable to include the contents of such substances as barley water when making the calculations for percentages.

Coconut water as a diluent has already briefly been noticed, and it would seem advisable to study this substance, as well as some others of local use, to ascertain their value and action upon the digestive tract of children.

There is now a voluminous literature on the employment of a solution of sodium citrate as a modifier of the curd in cows' milk used for infant feeding and its great value in this respect has been amply demonstrated. It is not generally employed in the Philippines and we would urge physicians to look more carefully into its merits. Limewater while an excellent modifier of the cows' milk curd in certain cases, is too extensively given and is often employed in too great a concentration.

IV. DIET.

Facts are expressed conservatively in stating that for the last four years an average of 2,500 children under 1 year of age have died annually in the city of Manila for want of sufficient or proper food.

The elucidation of the technical problems in this connection is for members of the medical and chemical professions. A study of the available supply of infant food in Manila is necessary and in the preceding pages an effort in this direction has been made, but the weapons provided by this discussion are to be used by all the people. A successful administration looking to the improvement of conditions can only be furnished by extensive organization and education. Members of our profession in Manila are often indifferent in questions of infant feeding. This is shown by the fact that there are but *very* few records of examinations of milks, either human or animal, to determine their nourishing properties, to be found in the laboratories of the city.

There is, therefore, entirely too much left to chance in the composition of the foods recommended and not enough adjustment of food values to special conditions as they are represented by individual patients. Directions should be very specific and given in writing. It is not enough to order a mixture of condensed milk and limewater to be given from a boiled bottle. The average, or even the most ignorant, mother knows how to prepare such mixtures herself, as she can read the directions on the label of the can of milk. We must far more closely study each infant and order a food which will properly nourish the child or remove the abnormal condition.

The service of one of us at St. Paul's Hospital has shown some of the most serious of errors in very general use, one which we wish especially to call attention to, namely the use of excessive quantities of limewater as a diluent. This is a very common practice, and many of the patients suffering from proteid starvation and chronic diarrhœa, marasmus, gastric atony, etc., are more apt to have the trouble caused by an exces of lime than by any inherent difficulties in the milk administered.

The preparation of infant foods for all kinds of the many conditions encountered is a difficult task, which can best be undertaken by milk laboratories similar to those now in use in many American and European cities. This procedure would cost a considerable amount of money, but with organization and education, funds might become available either through the Government or by gifts from philanthropic sources.

A discussion of the conditions confronting us in Manila together with analyses of food stuffs has been given in chapter three, and in order to give a more clear understanding, the following Table No. 15, representing a majority of the varieties of food supply obtainable in Manila, is inserted.

TABLE No. 15.—*Showing the essential facts taken from analyses of samples of milks and other most generally used infant foods available in Manila together with some normal working standards.*

Substance.	Fat.	Milk sugar.	Total proteid.	Casein.	Lact albumin.	Soluble carbohydrates.	Insoluble carbohydrates.
Average human milk (Rotch) [a]	3.00–4.00	6.00–7.00	1.00–2.00	0.59	1.23	------	------
Average human milk (König) [a]	3.80	6.20	2.30	1.00	1.30	------	------
Average milk, Caucasian woman, Manila.	4.24	6.63	1.45	------	------	------	------
Average milk, Filipino woman—							
Of better class, Manila	5.38	6.83	1.45	------	------	------	------
Of lower class, Manila	3.07	7.98	1.29	------	------	------	------
Average cows' milk (Rotch) [a]	4.00	4.75	3.50	2.66	.84	------	------
Average cows' milk (König) [a]	3.70	4.90	3.50	3.00	.50	------	------
Average cows' milk, Manila	2.82	4.21	3 15	4.70	------	------	------
Average goats' milk (Rotch) [a]	4.30	4.00	4.70	------	------	------	------
Average goats' milk (König) [a]	4.80	4.40	4.30	3.20	1.10	------	------
Average goats' milk:							
Native, Manila	5.73	3.54	4.05	------	------	------	------
Maltese, Manila	4.75	4.27	3.23	------	------	------	------
Average carabao's milk	10.65	3.73	6.31	------	------	------	------
"Bear milk"	4.27	4.06	3.07	------	------	------	------
"Dragon milk"	4.12	4.35	3.11	------	------	------	------
"Weribest milk"	3.24	4.75	3.10	------	------	------	------
"Bear cream"	38.07	3.27	2.29	------	------	------	------
"St. Charles evaporated cream"	7.96	11.34	8.21	------	------	------	------
"Highland evaporated cream"	7.86	11.13	8.40	------	------	------	------
"Sego evaporated cream"	10.08	8.29	6.95	------	------	------	------
"Tulip evaporated cream"	10.91	9.20	7.14	------	------	------	------
"Bear concentrated milk"	9.67	9.79	7.84	------	------	------	------
Australian (cold storage)	14	13.52	9.31	------	------	------	------
"Pet evaporated cream"	9.75	9.91	7.14	------	------	------	------
"Cow's head evaporated cream"	10.33	12.19	8.10	------	------	------	------
"Ideal evaporated cream"	10.10	11.03	7.36	------	------	------	------
"Nestles condensed milk"	10.62	12.53	7.90	------	------	[b]40.56	------
"Eagle condensed milk" [a]	8.44	11.69	7.23	------	------	[b]41.52	------
"Milkmaid condensed milk"	9.63	11	4.33	------	------	[b]45.88	------
"Gold seal condensed milk"	8.25	9.75	7.46	------	------	[b]57.56	------
"Perfection condensed milk"	.50	12.06	8.54	------	------	[b]50.10	------
"Meodora condensed milk"	4	11.55	8.70	------	------	[b]48.32	------
"Allenbury's food No. 1" [a]	14	------	9.70	------	------	[b]66.85	------
"Allenbury's food No. 2" [a]	12.30	------	9.20	------	------	[b]72.10	------
"Nestles milk food" [a]	4.45	------	10.72	------	------	[b]43.84	[c]35.34
"Mellins food" [a]	.30	------	10.10	------	------	[b]82.06	------
"Carmicks soluble food" [a]	2.18	------	16.60	------	------	[b]38.21	[c]38.54
"Imperial granum" [a]	.72	------	13.77	------	------	[b]3.94	[c]67.46
"Ridge's food" [a]	.48	------	13.83	------	------	[b]5.02	[c]69.24
"Health Food Companies barley" [a]	.89	------	6.98	------	------	------	[c]80.35
"Robinson's patent barley" [a]	.41	------	7.46	------	------	[b]2.91	[c]78.66
"Borden's malted milk" [a]	5.41	------	14.04	------	74.31	------	------
"Horlick's malted milk" [a]	1.41	------	14	------	63.87	[b]15.68	------
Oat meal water (Rotch) [a]	.08	------	.41	------	------	------	[c]2.99
Do. [a]	.11	------	.47	------	------	------	[c]4.58
Barley meal water (Rotch) [a]	.02	------	.54	------	------	------	[c]2.88
Do	.04	------	.68	------	------	------	[c]4.43
Fat free cows' milk (Rotch) [a]	.05	5	3.55	------	------	------	------
Whey from cows' milk (Rotch) [a]	------	5.10	------	------	.90	------	------

[a] Analysis not made in this laboratory. [b] Cane sugar. [c] Starch.

It has been shown that fresh milk is practically at present eliminated from our armamentarium, and with this fact before us text-books on infant feeding become almost valueless just at the point where our problem begins. This condition is of course largely responsible for the confusion, each man being to a great extent thrown upon his own resources.

The most generally used foods among the better classes have mainly been the so-called evaporated creams, diluted four to ten times with water, with perhaps the addition of an indefinite amount of limewater and sugar. When diluted sufficiently to give a suitable proteid percentage, the fat and sugar are far below the requisite amount. While the addition of milk sugar or cane sugar makes up one of the deficiencies, the fat percentage remains low, and as a result constipation and other defects of nutrition are very frequently observed. In many instances various mixtures of milks and of foods containing starch and sugar are concocted; these overcome the constipation, but they still leave the fat deficient, or the proteids too high and thus other and more serious disturbances arise.

Inasmuch as conditions are such as to make the employment of preserved milks imperative in the feeding of infants, it is necessary to use these substances to the best advantage. To accomplish this end our experience has shown us that it is better to disregard the claims and directions of the milk manufacturers, to analyze their products carefully and by careful combinations to *institute percentage feeding with the use of these artificial foods.* It is remarkable what may be done in this way with patients presenting the greatest difficulties in feeding.

However, in order to secure the best results with this percentage feeding of artificially prepared milks a most important prerequisite is constantly to impress upon mothers and nurses the necessity of the supervision of the physician. It is justifiable to explain to the mother that feeding of the infant is a more strictly medical matter than is even medical supervision at the birth of the child. Absolutely nothing should be fed except what is prescribed *in writing* by the physician and if members of the family are taught this from the birth of the infant and their confidence is assured by a carefully prepared formula for feeding, but little difficulty will be experienced in controlling the situation and insuring the health of many babies who would otherwise perish from dietetic errors. For this percentage feeding in Manila our armamentarium consists principally of the following substances:

1. Plain, sterilized, uncondensed milks: There are five brands on the market.
2. Pure creams: There are three brands on the market.

3. Sugars: Lactose and saccharose.

4. Diluents: The usual varieties of barley water, oat-meal water, lime-water and sodium citrate solutions.

5. Peptonized milks, butter milks, fat free milk and the other usual modifications of fresh milk may nearly all be made in a fairly satisfactory manner from the fresh, sterilized milks mentioned above by the use of appropriate ferments and other methods.

6. In place of the plain sterilized milks, condensed whole milks or the so-called evaporated creams which are in reality only condensed whole milks, may be used provided the percentage method of dilution is used and the deficient fat made up by the addition of cream.

7. In special cases and where price is an object, the condensed sweetened milks may be used by following the percentage methods in dilutions and by the addition of cream to replace the deficient fat.

All the milks which have been mentioned have been carefully analyzed either in this laboratory or by other authorities and the averages shown in Tables Nos. 11 and 15 may be used as sufficiently accurate for practical purposes.

By using these averages and figuring on 100 cubic centimeters of finished product as a percentage basis, calculations are easily made and the only vessel necessary in preparing the food is a clean, graduated, glass cylinder; one of 1,000 cubic centimeters capacity will be found most useful.

The rich, tinned creams do not keep very well after the can is open and for that reason it is well to prepare an entire day's feeding at one time, fill it into the necessary number of clean nursing bottles, plug with cotton and keep in the ice box until ready for use.

Some prescriptions for percentage feeding are given below, but in order to make the subject still more clear the following example is submitted.

Suppose it is desired to give an infant 1½ months old, eight feedings of 100 cubic centimeters each in twenty-four hours, each feeding to contain fat 4 per cent, sugar 7 per cent and proteid 1 per cent. By simple calculation or by reference to the Cox chart number 1 we find that 1 per cent proteid and 4 per cent fat may be obtained by using 24 cubic centimeters of one of the above-mentioned sterilized milks and 11 cubic centimeters of one of the 27 per cent creams or 7½ cubic centimeters of the 38 per cent cream to each 100 cubic centimeters of finished product, or 192 cubic centimeters of milk and 88 or 60 cubic centimeters of cream for the entire day's feeding of 800 cubic centimeters. Simple calculation shows that this substance will contain but 1.6 per cent of sugar and this deficiency may be made up by the addition of 5.4 grams of milk sugar per 100 cubic centimeters of finished product.

Having determined the necessary amounts of the different substances to be used in the finished product, a prescription should be written showing these amounts and the percentages of each ingredient. The mixture is best made in the following manner:

Place the necessary amount of distilled or boiled water in the graduate, add the milk sugar and stir with a clean glass rod until solution is complete, next add the requisite amounts of milk and cream and stir again. Finally, add the limewater, sodium citrate or other modifier, stir and pour the product directly into the necessary number of clean nursing bottles, stopper with clean cotton and place in the ice chest until ready for use.

When using sodium citrate in the food it is best to prescribe a 3 or 5 per cent solution to which a few drops of chloroform have been added to prevent bacterial decomposition.

With little care, any reasonable percentage food may be made according to the above method and we need not at this day emphasize the confidence with which such known percentages are fed, nor the excellent control which a knowledge of *just* what the infant is being fed gives us over many of the more frequent disturbances which food often causes in infants.

Both the manner of percentage feeding, and its results may be further shown by a few illustrative cases.

CASE 1.—*Four months old, weight 9½ pounds, anœmic, wasted, diarrhœa with excoriations about anus, mucous membranes of mouth red and inflamed, abdomen prominent, vomiting of food, fretful and crying much of the time. Percentage feeding instituted with rapid gain in weight and complete recovery.*

This baby was a normal child at birth, weighing 8 pounds. The mother's milk was reported to be deficient in quantity from the beginning but no examinations were made. At 2 weeks of age alternate feedings of breast milk and a certain condensed milk were instituted, the milk being prepared according to the directions of the manufacturer. No improvement followed and at 5 weeks of age another milk was tried. Still the baby failed to improve and during the next ten weeks the giving of still another brand of artificial food was instituted. When the baby came under our observation a dose of castor oil was given and all food withheld for twenty hours, after which a prescription was given of sterilized milk, pure 27 per cent cream, milk sugar and sodium citrate in such proportions as to give a little less than the normal percentage of fat, sugar and proteid. The child enjoyed the food, did not vomit and improvement was rapid from the date the feeding was instituted. By altering the amounts of "milk" and "cream" which were used, the percentages of the fat, sugar and proteid were changed so that at 5½ months of age the child was taking normal amounts and was in excellent health; he passed from observation at 8 months of age. A study of the mother's statements about the amount and kind of food previously given in connection with the analysis given in Table No. 15 shows that this baby had been receiving too much proteid (3½ per cent) and sugar (8 per cent) and not enough fat (1.8 per cent), and in addition 10 per cent limewater was being used.

CASE 2.—*Four months of age, weight 12 pounds, fretful and crying, fontanelles sunken, muscles wasted, constipation, head perspiring, tender joints and back,* *takes food irregularly, followed occasionally by vomiting. Percentage feeding instituted with rapid recovery.*

Because of failure of breast milk this normal 8½-pound baby was given substitute feeding at 3 weeks of age consisting of an "evaporated cream," prepared according to the directions of the manufacturer. There was no improvement and when the infant came under observation it was receiving a one-fourth dilution of this milk plus 10 per cent limewater and 5 per cent milk sugar. Figuring from our Table No. 15 we see that this baby was taking about as follows: fat 2.5 per cent, sugar 9 per cent, and proteid 2.75 per cent. The proper percentages were instituted here by simply combining a 38 per cent preserved cream with a *proper amount* of the same excellent food the baby had been receiving, with the addition of the necessary sugar and the changing of the limewater to a solution or sodium citrate.

The trouble with this patient as with the other and many more whom we could enumerate, was the lack of proper proportions of the essentials in the food, and this lack was largely due to the want of appreciation of what was necessary and to a manufacturer's claims for the superiority of his milk.

By proper adjustments of amounts of the various brands of sterilized, pure cow's milk, evaporated or condensed milks and preserved and condensed creams, a fairly wide variation of percentages may be made and it is in this way that we may obtain the best results from feeding preserved milks. What is most needed on the part of manufacturers is the giving of more accurate information concerning the composition of their foods and their methods of preparation, with fewer statements about what they will accomplish. Special results in special cases are more the result of elasticity in the babies' metabolism than in any unique composition which may be given to any one brand of cow's milk evaporated *in vacuo.*

Chart No. 1, opposite this page, which has been prepared for us by Dr. A. J. Cox, Chemical Laboratory, Bureau of Science, taken in conjunction with Table No. 15, will help the physician in calculating his percentages from the principal milks to be found in the Manila market:

Explanation of Chart No. 1.[5]—The fact that children of different ages require milks of different composition for their proper nourishment, makes it very desirable that we have some way of quickly ascertaining the proper mixture of milk and cream and its dilution. The old cut and dried method is unsatisfactory and extremely slow because of the great number of variable factors involved.

The very close agreement between the analyses of all the brands of pure, sterilized milks for sale on the Manila market makes it possible to prepare a chart from which the amounts of lactose, water, milk, and cream which will give the proper percentage of constituents in the final product, may be calculated when any one of these milks and any standard pure cream are used. The chart is prepared on the basis of a 38 per cent cream, but as is explained blow it is equally well adapted for the

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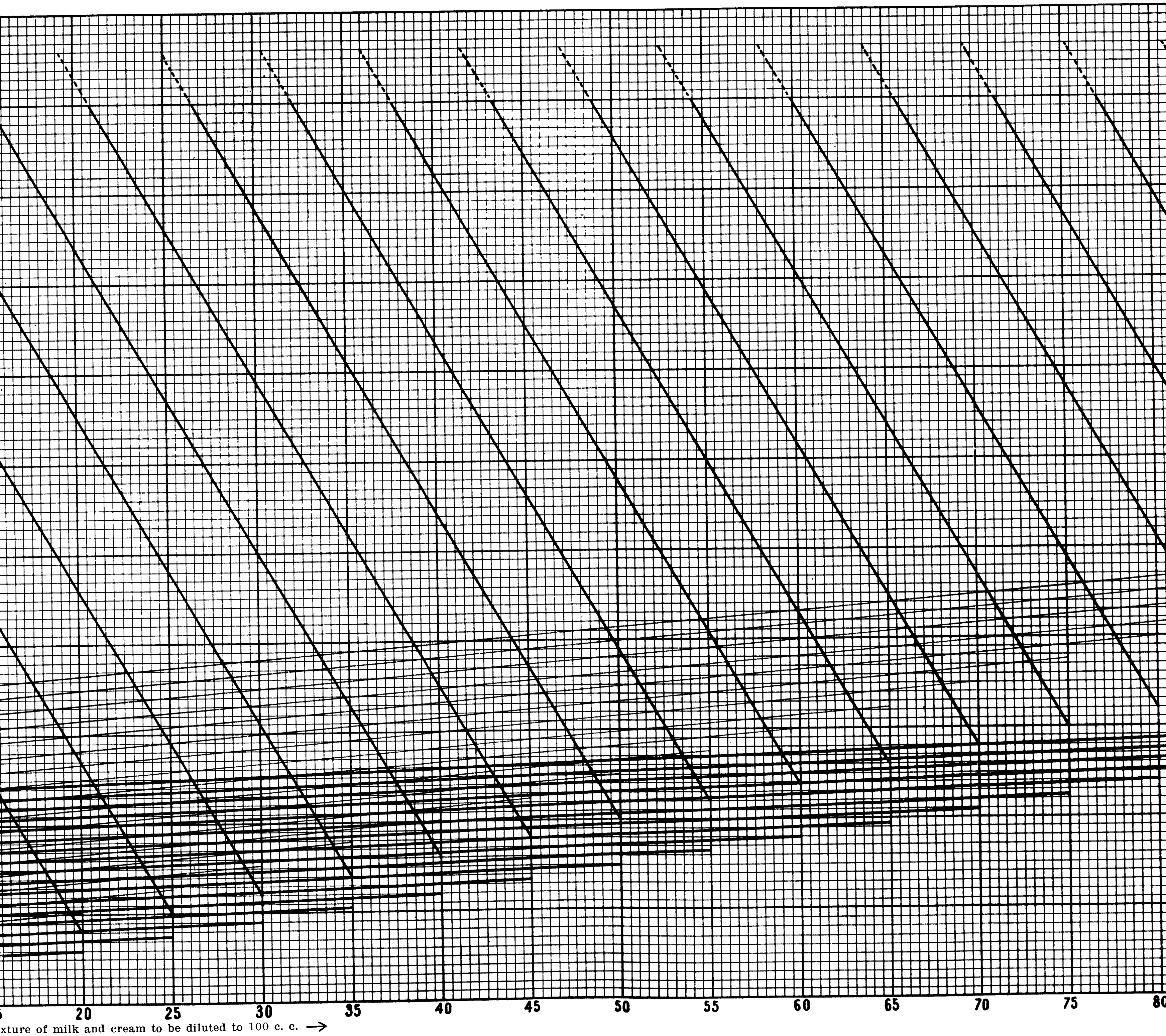
[5] By Dr. A. J. Cox.



xture of milk and cream to be diluted to 100 c. c. →

e curves to the reader's right.

chosen ordinate (vertical line) shows the number of cubic centimeters of milk and from this ordinate to the end of the curves shows the number of cubic centimeters of cream. These num

d, sugar, and fat—are shown by the points where the chosen ordinate representing the relations between milk and cream cuts or intersects the proteid, sugar, and fat curves, respectively, wh

be diluted to 100 c. c.

CHART NO. 1.

27 per cent creams which are now on the market. The average of the pure sterilized milks[6] previously mentioned has been taken as—

	Per cent.
Fat	4
Sugar	5
Proteid	3

The average[7] of the analyses of several samples of the 38 per cent cream is as follows:

	Per cent.
Fat	38
Sugar	3.3
Proteid	2.3

It will be seen that the percentage of proteid is roughly the same in both milk and cream, so that for young children the desired low proteid content may be obtained simply by dilution of either of these, but this would not suffice for the content of fat. If milk alone were diluted the fat would be too greatly reduced and if the cream alone were used it would be too high, but by using both milk and cream a mixture may be obtained which will also give the desired fat content.

Enough sugar of milk may then be added to increase this constituent to the percentage desired: or better still, after the factors are established, dissolve the sugar of milk in the water before adding the milk and cream, for then one can see that a perfect solution is attained.

For the mixture of cream with milk the percentage of each constituent varies from that of the milk toward that of the cream directly in proportion to the amount of cream added, that is, a straight line joining the percentages of a constituent in the milk and the cream gives all the possible variations for the percentage of that constituent. Now, if this mixture is diluted with water we would get the same result divided by the amount of dilution, or a curve (straight line) parallel to the former with its lower end terminating on the number of cubic centimeters of the mixture which was diluted to 100 cubic centimeters. These results have all been expressed in the chart. In the chart, the curves have been made for variations of 5 cubic centimeters in the total mixture of milk and cream to be diluted to 100 cubic centimeters. Closer results could be obtained if 1 cubic centimeter variations had been plotted, but the network of curves would have been confusing. The curves as they are given only approximate the desired results, but they are accurate enough for all practical purposes.

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[6] If one of the sterilized pure milks is not at hand, a milk of approximately the same composition may be had by diluting a volume of any of the better class of concentrated milks, or "evaporated creams" shown in Tables No. 11 and 15, with 1½ volumes of distilled water, that is, every 40 cubic centimeters should be diluted to 100 cubic centimeters and this may be used instead. Any good quality of (Maltese) goat's milk may also be used.

[7] One sample which contained 20.8 per cent fat and 9 per cent sugar was not included in this average.

These being the data, the following example is explanatory: For an average child six weeks old we desire a milk of the following composition:[8]

	Per cent.
Fat	4
Sugar	7
Proteid	1

We may start with either the desired proteid or fat content. Let us take the proteid simply because these curves lie closer together and it is easier to differentiate at the beginning. For 1 per cent content we have two possibilities, either the curve which terminates in the 40 or the one in the 35 cubic centimeters of total mixture of milk and cream to be diluted to 100 cubic centimeters. Let us first consider the former. If we follow the fat curve for this mixture to 4 per cent, the ordinate at that point does not cross the proteid curve at the desired point, namely, 1 per cent. If on other hand we take the fat curve for the 35 cubic centimeter mixture and follow it to 4 per cent, we find the ordinate to cut the proteid curve at 1 per cent content, and this shows us the proper relation to be 27½ cubic centimeters of milk and 7½ cubic centimeters of cream. Now the sugar of milk curve which terminates on 35 cubic centimeters intersects the chosen ordinate 27½ at 1.6 per cent. This gives us the sugar content of the mixture of milk, cream and water. We desire 7 per cent. It is necessary then to add as many grams of sugar of milk as the difference between the desired and actual sugar content, namely, 7 minus 1.6 or 5.4 grams per 100 cubic centimeters. Therefore the desired amounts of each are as follows:

Water	cubic centimeters	65
Milk	do	27½
Cream	do	7½
Sugar of milk	grams	5.4

If a pure cream with a fat content other than that which is the basis of the chart,[9] namely 38 per cent, is used, then a correction in the quantities of milk and of cream as given above is necessary. If a cream with

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[8] Rotch, T. M.: Pediatrics (1906), 196.

[9] Two such samples have recently appeared on the Manila market namely "Natura" and "Butterfly Brand." The analyses of single samples of these made by this Bureau are as follows:

	Natura.	Butterfly.
Fat	27.1	26.95
Sugar	2.3	4.00
Proteid	2.4	3.51

a fat content lower than 38 per cent is used, then in order to give the desired composition the cubic centimeters of cream must be *increased* to

$$\frac{38-4}{(\text{The per cent of fat in the cream used})-4} \times \text{The number of cubic centimeters of cream given by the chart.}$$

and the cubic centimeters of milk *diminished* by the amount of the increase in the quantity of the cream. If a cream with a fat content higher than 38 per cent is used, then the cubic centimeters of cream must be *diminished* to

$$\frac{38-4}{(\text{The per cent of fat in the cream used})-4} \times \text{The number of cubic centimeters of cream given by the chart.}$$

and the cubic centimeters of milk *increased* by the amount of the decrease in the quantity of the cream. The proteid and sugar of all pure creams have approximately constant values, so that for these the chart is always applicable and no appreciable error is produced when the correction for fat is made.

Let us consider what the results of the above example would be were a 27 per cent cream to be used instead one of 38 per cent. The percentage of fat in the former is lower than it is in the latter, therefore the cream required would be increased to $\frac{38-4}{27-4} \times 7.5$ or 11 cubic centimeters, an increase of 3.5 cubic centimeters over the quantity of 38 per cent cream. The milk would be diminished by 3.5 cubic centimeters or reduced to 27.5 — 3.5 or 24 cubic centimeters. Hence, the desired amounts of each ingredient to give a milk of the above composition when a 27 per cent is used would be—

Water	cubic centimeters	65
Milk	do	24
Cream	do	11
Sugar of milk	grams	5.4

If it is desired to add lime water (sodium citrate, etc.) then the water content should be diminished by the number of cubic centimeters of lime water (sodium citrate, etc.) to be added. If more than 100 cubic centimeters of the mixture is desired, multiply the quantities of water, milk, cream, and sugar of milk respectively by as many times as the quantity desired is greater than 100.

GANGOSA IN THE PHILIPPINE ISLANDS.

By W. E. MUSGRAVE and HARRY T. MARSHALL.

OUTLINE.

I. Clinical report.
II. Autopsy report.
III. Résumé of literature.

Gangosa has not previously been reported for the Philippine Islands. Because of the comparative rarity of the disease, its limited geographical distribution and probably infectious nature, the following case is of sufficient importance to warrant discussion. The patient was seen several times by Dr. N. T. McLean, United States Navy, who has had a large experience with the disease in Guam, and the diagnosis was confirmed by him.

I. CLINICAL REPORT.

Case record.—Gangosa; death; autopsy.—The patient, C. F. (2179) was admitted October 31, 1906, to Dr. McDill's service in St. Paul's Hospital. He was transferred to Dr. Musgrave's service on December 15, 1906, and died January 23, 1907. He was a Filipino, male, 29 years old, single and a native of Santo Domingo de Basco, Batan Islands.[1] The patient was an unusually ignorant native of the lower classes and the value of his statements was not very great. However, the following facts obtained at different times and repeated to different interpreters are probably true. Father, mother, and two brothers are living and in good health. The patient stated that none of his relatives has a disease like his but he was equally positive that several other people in the same town are suffering from a similar condition. Previous diseases were denied. The patient stated that he was never far from his native town until he came to Manila two years ago and that he had never been ill except once with *calentura* (fever). The last two years before entering the hospital he resided in one of the barrios of Manila and worked as a stable boy most of the time.

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[1] The *Batanes* constitute the most northern group of the Philippine Islands. They lie about 120 miles due north of Luzon.

His statements about the time of the onset of his present illness differed very much, but Dr. Ariston Bautista y Lim, who saw him, thinks it was probably about five years ago that he first noticed sore throat and that the disease had been gradually growing worse since that time. He had taken many kinds of medicine during the last two years without any improvement. On admission to the hospital the patient was considerably emaciated and anæmic, the voice had the intonation peculiar to destructive conditions of the larynx and soft palate and there was an opening about 1 centimeter in diameter in the bridge of the nose. Upon closer examination it was found that the uvula was partially destroyed and the palate had two dirty, grayish-appearing, perforating ulcers. The pharynx, upper larynx, palate, posterior nasal structures, both bone and soft tissues, were partially destroyed by a chronic type of ulceration and there was a considerable amount of scar tissue, showing some evidences of repair. The margin of the upper lip was also destroyed, leaving a grayish, granular surface. The odor from these lesions was exceedingly offensive and there was at times a slight discharge of granular, necrotic material. Microscopic examination of fresh and stained smears, made by scraping the surfaces of the wounds, were negative for acid-fast bacilli and for treponema. During the period of observation the nasal wound increased in size, there was more deformity due to destruction of bone, and the destructive lesion on the upper lip progressed slowly until death. Plate I is from a photograph made about one month before the fatal termination. There was diminished sensation over the surfaces of the wound, which in places amounted to local anæsthesia.

The skin was otherwise rough but apparently free from disease or scars. About five weeks before death a dull, chronic-appearing skin ulcer developed on the outer side of the left shin and this continued slowly to extend until death, at which time it was about 1 centimeter in diameter and extended through the skin. The superficial lymphatics were slightly enlarged in the groins, but not more so than is commonly seen among the lower classes of barefoot people here. The muscles were wasted, the joints were free from disturbance and except as above noted, no bone lesions could be found. No abnormalities were found in the circulatory system except an anæmic murmur of the heart. Temperature was normal and there was but slight fever at any time during which the patient was under observation, which was nearly three months. Two days after admission there developed an irregular, intermittent fever varying between normal and 38°.5, which lasted for a few days, and again, just preceding the fatal termination, there was slight pyrexia. A blood examination on admission showed no leucocytosis and no abnormal elements. A blood examination five days before death showed no malaria; leucocytes 11,600, hæmoglobin 80 per cent.

Differential count.

	Per cent.
Small lymphocytes	10.0
Large lymphocytes	4.6
Polymorphonuclears	82.0
Eosinophiles	2.6
Basophiles	.4

There was slight cough at times during the patient's stay in the hospital, but it was never annoying and the expectoration, if any, was swallowed. A physical examination of the chest on admission showed signs of some bronchitis. There were no subsequent examinations. The sputum was not examined because none could be obtained. The tongue was constantly coated with a heavy, brownish fur, but no ulceration took place. The appetite was poor, the patient had to be fed largely by a tube, there was no vomiting or pain. The bowels were constipated, moving but seldom except from cathartics or enemas. Microscopic examination of the feces showed ova of *Trichuris trichiura.* There were no disturbances of the urinary function. Examination of the urine showed a trace of albumin and a few hyaline casts. This patient never complained of pain; as a rule sleep was sufficient, but during the last week of his illness there was considerable restlessness and insomnia. The reflexes were normal. The eyes were apparently normal, but hearing became somewhat defective toward the end. The sense of smell was almost *nil* and speech was of the Gangosa type, its peculiarity resulting from destruction of the palate.

Treatment.—A probable diagnosis of syphilis was made when the patient was first admitted to the hospital; antisyphilitic treatment was instituted and continued for three months. This treatment consisted of intramuscular injections of mercury to the point of toleration, of potassum iodide by mouth and intravenously in doses up to 10 grams per day. Mercury was also tried by inunction.

The antisyphilitic treatment did not cause any improvement and it was discontinued after three months. This was followed by copper sulphate in 0.015 doses, three times a day for about one month without any improvement and no further specific therapeutic measures were undertaken. Death occurred January 23, 1907. *Clinical diagnosis, Gangosa.*

II. AUTOPSY REPORT.

Autopsy.—Four hours after death. The body is markedly emaciated, rigor mortis of moderate grade present, one old, grayish colored ulcer on the left leg about 8 millimeters in diameter extends to the deeper layers of the skin. The muscles are wasted and pale. The *superficial lymphatics* in the groins moderately enlarged, pale in color and quite firm.

The *left lung* contains a few, reddish-colored infarcts from 7 millimeters to 1 centimeter in diameter, mostly in the lower lobe. There is an old, adhesive pleuritis on the right side; the *right lung* contains several areas similar to those in the left and there is also a small tuberculous cavity in its apex. There is a moderate bronchitis, and a few areas of beginning broncho-pneumonia in both organs. Nothing abnormal is found in the *heart* or its membranes.

The *abdomen* is free from adhesions, the tissues and organs appear to be anæmic and with slight enlargement of some of the mesenteric lymphatics. No marked departure from the normal is seen in any of the organs except the *stomach.* This shows a general hemorrhagic catarrh of the mucosa.

In order to preserve good museum specimens, the soft tissues of the neck were divided at the upper end of the sterum, carefully dissected up from the cervical column and the latter cut by sawing through the first cervical section. The brain was removed, but nothing abnormal was found in this organ. The rest of the head and soft tissues of the neck were placed in formalin for two days and then, after hardening, they were divided antero-posteriorly in the median line. By this method a careful study of the lesions of both bone and soft tissues was made possible.

In these specimens a condition typical of that described for gangosa is found. There is marked destruction and some scar formation of the soft parts, including the larynx, pharynx, soft palate, uvula and nasal tissues. The nasal septum and a considerable area in the bridge of the nose, including both bone and cartilage, are destroyed. The palate is also partially destroyed. The process in addition has extended into the sinuses of the cheek bones and here it has included both bone and soft tissues. There is no evidence of acute inflammation, the surface of the destroyed areas is covered with a dirty, granular material, which when removed leaves a gray, sponge-like tissue. Scrapings from the diseased areas are free from acid-fast bacilli and treponema.

Smears from the pneumonic areas in the lungs showed a single filarial embryo but no others were observed during the examination of many preparations from similar locations.

Anatomic diagnosis.—Chronic ulceration of the naso-pharyngeal tissues including destruction of the palate, nasal septum, turbinates and involvement of the maxillary sinuses; together with extensive destruction and scar formation of the adjacent soft parts of the larynx, pharynx and nasal tissues. Tuberculosis of the right lung with a small cavity in the right apex. Terminal broncho-pneumonia. Moderate lymphadenitis, more marked in the region of the neck. Acute hæmorrhagic catarrh of gastric mucosa.[2]

[2] The only other autopsy which has been made upon a patient with gangosa is one reported by Mink and McLean. In their case, a male of 18, the entire nasal passages, hard and soft palate were destroyed; the tonsils and pillars were replaced by scar tissue; there was cardiac hypertrophy, and fine adhesions existed between the lungs and pleuræ. The diagnosis in their case was "native epidemic asthma."

HISTOLOGY.

Ulcer I, edge of skin, hæmatoxylin and eosin.—On surface of ulcer there is a structureless, necrotic membrane, cloudy and purplish, in which one can dimly make out degenerated cells. Necrotic processes extend from this membrane into clefts in the tissue beneath. The transition is sharp between the necrotic membrane and the subjacent, richly cellular tissue. The latter is a loose, vascular, areolar tissue containing a moderate amount of fat and supported by heavy trabeculæ of connective tissue. A few strands of degenerating voluntary muscle are found in the deeper part of the section. The loose fatty tissue is black from accumulations of small round cells, which are abundant just at the necrotic border and diminish in numbers as the distance from the edge of the ulcer increases. Mingled with the small round cells are a few large mononuclears, larger epithelioid fibroblasts, and numerous plasma cells with small, eccentric, dark nucleus and fairly abundant irregular bluish protoplasm.

The necrotic process is evidently advancing and has already involved several vessels. One of these, a small artery, is plugged with a hyaline mass and its walls can no longer be made out because of the dense, small, round cell infiltration, but near by a wide, thin-walled sinus shows no obliteration at all. With the exception of the small artery above mentioned, and one other artery near the advancing edge of the ulcer, in which the same process is occurring, there is no special accumulation of round cells around the vessels.

The muscle in the deeper part of the section is undergoing necrosis, and in large measure has become converted into a homogeneous or finely granular, bright-red mass, with fine nuclear fragments scattered through it.

At the edge of the ulcer there are two irregular downgrowths of stratified epithelium, one nearly a millimeter in length. In each of these areas the upper layers are of irregular thickness and composed of large, poorly staining cells, often vacuolated, in which the nuclei show various irregularities. The deepest layer of cells is as a rule uniform in arrangement and staining, the downgrowths being no more atypical than those in a benign papilloma of the skin. However, along one side there are several delicate, finger-like projections of epithelium entering directly into the underlying tissue, the protoplasm extending beyond the last nucleus to form a protoplasmic mesh work in which several small, round cells are gathered. The arrangement makes it difficult to determine exactly where the epithelium ends and connective tissue and infiltrating cells begin.

One of these epithelial areas is definitely retracted from the adjacent tissue, and this shows no downgrowths.

There are none of the irregular nuclear figures nor epithelial pearls which occur in epithelioma and the nuclei of the cells of the deepest layer of epithelium remain fairly characteristic, in spite of the irregular protoplasmic downgrowth. It is to be noted that the nuclei of the epithelial cells do not invade the underlying tissue, the downgrowth being altogether protoplasmic.

Sections B, C, D, E, F, and G, taken from different parts of the ulcerating area between the anterior nares and the pharynx, have much the same appearance, and are characterized by advancing necrosis with very little reaction on the part of the tissue. The necrosis is advancing upon areolar tissue, upon mucous glands, voluntary muscle, etc. In only one case is there any decided reaction, and that is found in a section taken evidently at the junction of the ulcer and face. The section passes through the edge of ulcer and adjacent skin. At a distance from the ulcer the epithelium is delicate and thin, pigment is abundant, the deepest layer of cells is uniform, the under surface of the epithelium is fairly even, with only a few small papillæ. The hair follicles, sweat glands, sebaceous glands and subcutaneous tissue appear normal. As the edge of the ulcer is approached, the subcutaneous tissue becomes slightly œdematous and finally infiltrated with cells. The epithelium becomes thicker and thicker, the horny layer reduced, the epithelial pegs longer, thicker and more irregular, and the pigment disappears. Just at the edge of ulcer the epithelium dips down as an irregular peg, 1 millimeter into the subcutaneous tissue. The epithelial cells are large, cloudy and pink, often vacuolated, the nuclei are vesicular, pale, uniform and do not show the irregularities met with in epithelioma.

Over the ulcer there is a thin layer of necrotic, granular, pinkish material, beneath which lies a narrow zone of disintegrating cells, red blood corpuscles, etc., immediately followed by a richly cellular and vascular granulation tissue. In this tissue there are many capillaries, round and elongated fibroblasts, disintegrating red blood corpuscles and much yellow blood pigment, and a fairly large amount of fibrillated intercellular material. In the outer border of the granulation tissue there are a small number of lymphocytes and cells with small, round, dark nuclei and fairly abundant pink to purple protoplasm, the nucleus being sometimes central, sometimes eccentric.. Extravasated red corpuscles and yellow blood-pigment are found as far as the deepest limits of infiltration. There is no evidence of perivascular infiltration. There are no polymorphonuclear leucocytes, excepting at the necrotic edge of ulcer.

The granulation tissue is less than 2 millimeters at its thickest part. There is an abrupt transition from this to the slightly œdematous, fatty subcutaneous tissue. Within 2 to 3 millimeters of the surface of the ulcer two large arteries are found with their walls very œdematous, and the lumina becoming obliterated. At some distance below the end of infiltration there is found a strand of young granulation tissue and plasma cells, but there is no evidence of the occurrence of young foci of necrosis beyond the advancing edge of the ulcer. This section differs from the others in the relative abundance of granulation tissue. In the other sections there is very little granulation tissue formed.

In sections stained by the Weigert-Gram method and for tubercle bacilli there is nothing characteristic. No acid-fast bacilli are found. There are a number of cocci, mostly diplococci, and a few slender, blue-staining curved rods on the free surface of the ulcer; elsewhere no bacteria.

The heart.—Stains well; the structure is well preserved; the epicardium slightly œdematous; in the meshes are scattered a few small, round cells and occasionally, cells with red protoplasm; the muscle fibers also are quite widely separated, the intervening spaces being clear or containing small amounts of pale, pink, granular or fibrillated material but no leucocytes. The vessels are normal and show no perivascular leucocytosis.

The protoplasm takes a bright eosin tint; striation and fibrillation are distinct; the muscle nucleus is surrounded by an oval, clear space two to five times the length of the nucleus, containing only a slight amount of brownish pigment. This central clear space often makes the fibers which are cut on longitudinal section appear to be split, and in general the muscle fibers are drawn out into longer and narrower ribbons than usual. The nuclei are small, fairly uniform, pale and vesicular with a darker nuclear capsule and a variable number of chromatin dots. A moderate number of fibers show myocardial fragmentation with the stair-like arrangement of fibrils.

Lung, hœmatoxylin and eosin and tubercle stain.—Typical, acute broncho-pneumonia is shown in one section. Some alveoli are nearly clear; others contain coagulated albumin. Congestion, œdema and desquamation also occur; in other places there are consolidated areas in which the alveoli are packed with polymorphonuclear leucocytes, red blood cells and fibrin in varying amounts, fibrin being unusually abundant. A bronchiole cut in the section has its wall infiltrated with polymorphonuclears and its lumen stuffed with them, together with a few desquamated cylindrical cells.

In another block the lung tissue has broken down forming a small abscess.

Section through another nodule presents a different appearance. Here the process answers to the description of disseminated tuberculosis of the lung. There are discrete and confluent miliary nodules, surrounded by consolidation beyond which is more or less normal lung tissue.

In the center of a miliary nodule is a nearly concentric whorl of coarse, red, coagulated fibrillar material. Surrounding this center is a zone of more loosely lamellated fibrinous material, in the meshes of which are a moderate number of epithelioid and small, round cells. There is no sharp demarcation between this zone and the necrotic center, and no clear zone of nuclear disintegration except in an early stage, and even then the nuclear fragments are diffused through the whole nodule and there is no karyolysis. Dividing connective tissue nuclei are found in the outer part of this zone, together with numbers of large cells with a single eccentric vesicular nucleus, and fairly abundant protoplasm which takes a bright eosin stain. At the outer margin of the central core, giant cells are occasionally found, with a dozen or more vesicular nuclei placed murally or at the poles of the cell, the protoplasm being abundant, irregular in outline and of a bright red color.

New capillaries and connective tissue cells are abundant between the nodules, the new vessels often being engorged. The nodules contain but few polymorphonuclears. Apparently the nodules are grouped in the neighborhood of a dilated bronchiole.

There is very little pigment deposited along the lymphatics, but disintegrated red corpuscles and blood pigment are found near the foci of coagulation necrosis.

In a section of this blook stained with carbol-fuchsin and methylene blue a small number of slender, acid-fast bacilli are found, often beaded, and occasionally slightly branched.

Liver.—Sections of liver stain well; there is considerable irregular contraction and shrinking of the parenchyma cells, especially around the hepatic veins. The parenchyma cells show no pigment, they are coarsely granular and stain deeply in eosin, and are very irregular in size. There is no evidence of cellular infiltration, and no new connective tissue, even where the shrinkage of parenchyma cells is most marked.

Spleen.—The section stains well; capillaries are moderately dilated; the contents in many cases have fallen out; the trabeculæ are somewhat loose and spongy, especially at their margins; the Malpighian bodies are inconspicuous, reduced in size and possibly in number. The sponginess of their reticulum is one factor reducing the prominence of the Malpighian bodies. The bodies contain normal appearing lymphocytes; there is no proliferation. Occasionally, in the center of the Malpighian body, there are found several smaller or larger blocks of bright, red, structureless hyaline material, irregular in size and shape, and apparently free in the tissue. In the neighborhood of such material it is common to find a few of the mononuclears with an increased amount of protoplasm which also has a bright, hyaline-red appearance and which crowds the nucleus to the side.

A focus is found in the pulp resembling an encapsulated solitary tubercle, 2.5 millimeters in diameter. The center is composed of a mass of reddish detritus, mostly granular, with a few fragmenting nuclei. At the periphery is a narrow zone of young connective tissue, advancing toward the necrotic center, apparently devoid of vessels. Surrounding this is a narrow zone of concentric lamellæ, resembling connective tissue, in the loose meshes of which are rows of small, round cells, usually having irregular nuclei and a few adult connective tissue cells. This zone merges at once into the pulp.

The endothelium of the small arteries is often thickened and irregular, and takes a bright-red hyaline color; the thickening is often uneven, and confined to one-half or less of the circumference of the vessel. This hyaline material appears to be amyloid in the hæmatoxylin and eosin stain, but it does not respond with iodine, iodine and sulphuric acid, nor with methyl violet stains. There is no increase in small, round, cells, nor in polymorphonuclear leucocytes.

Kidney.—The capsule is not thickened; the capillaries and veins are filled with red blood corpuscles; the glomeruli are well preserved, moderately congested, the cells not increased, the capsules of Bowman delicate and normal. The convoluted tubules contain a small amount of granular débris and a fine meshwork of coagulated matter; the lining cells are large, granular, bright red and frequently send protoplasmic fringes into the lumen; the nuclei are near the basement membrane and apparently normal. The loops of Henle and the collecting tubules appear normal. The interstitial tissue is not increased. There are no infiltrating cells.

Pancreas.—The parenchyma cells have retracted from the stroma producing distortions. The cells are small, and rather deeply stained. The islands of Langerhans are normal.

An area is found, miliary in size, having the characteristics of an early tubercle. The nuclei in this area are small, black, fragmenting and irregular; the protoplasm bright-red and hyaline. The area is not sharply marked off from normal pancreas. No karyolysis, no cellular infiltration.

Cervical lymph gland.—In one section the blood vessels are moderately engorged; the lymph channels are widely distended but nearly empty; the stroma is loose and the strands widely separated; the cell nests are not seen, and the cell columns are less dense than normally.

Two areas of necrosis over 1 millimeter in diameter are found. In each case the nodule consists of a large, central mass of bright-red detritus, granular and lumpy, with a few fragmenting nuclei, surrounded by a narrow and imperfect zone of large epithelioid cells with considerable dark-red protoplasm, irregular in shape, and much red-staining interstitial tissue. This zone is free from capillaries and passes over suddenly into lymph gland tissue.

In the peripheral sinus only a few cells are present; several of these are small, with deeply staining nucleus and a narrow band of red protoplasm, taking much the same shade as the erythrocytes. The cells in the internal sinuses are (1) small round cells and (2) large cells with vesicular nucleus and abundant, bright-red protoplasm, apparently desquamated endothelial cells. Many of these are cloudy and have only faint traces of the nucleus remaining. In some cases no nucleus is seen.

In another section the structure of lymph gland is better preserved. The cell nests and cell strands are clearly marked, in contrast to the dilated sinuses which contain many large, pale cells like those described above. There is no endothelioid cell proliferation at the center of the cell nests.

In a section stained with Ehrlich's triacid stain and examined with the oil immersion, the large cells in the sinuses take a diffuse, reddish-purple tint, and show no granulation.

Tissue taken from various parts of the ulcerating area in the nares and prepared by the silver impregnation method of Levaditi do not show spirochætæ, nor other characteristic organisms.

Histological diagnosis.—Necrosis of the nasal passages and adjacent parts; tuberculous nodules in lymph gland, lung, spleen and pancreas, hyaline and amyloid (?) degeneration of spleen; acute bronchopneumonia.

REMARKS.

The only other histological study of this disease which has come to our notice is that of Fordyce. He removed bits of tissue from the edge and center of the ulcer of his case, and made careful studies of specimens stained with various dyes. Although his case resembles ours in many respects, there are several points of difference. In both cases there was an ulcer bordered by tissue showing an infiltration chiefly with small round cells, but also showing numerous plasma cells. In his case there were but few polymorphonuclear cells; in ours such cells were difficult to find. Giant cells, which were fairly numerous in his sections, were absent from ours, although in one section there were proliferating capillaries, which sometimes took the form of a long, vacuolated cell with two or three nuclei. There were no true giant cells in our other sections from the ulcer. In neither case was there caseous degeneration, but in ours, even below where the cellular infiltration was marked, there was diffuse, advancing degenerative change in fibrous, glandular and muscular tissue, which was not present in Fordyce's case. In each, there was hæmorrage into the tissue, which was quite a striking feature of our case, while new forming capillaries, abundant in his case, were noticeably absent from our sections, except under the ulcer at the junction with the skin of the face. Parakeratosis and acanthosis were noted by Fordyce. The epithelial changes in our case, and described above, were similar, but there was not an increase in the horny layer, which was in fact diminished. The epithelial downgrowth in Fordyce's case gave a picture resembling epithelioma (Plate I), a condition which we could definitely exclude.

Summary.—The main differences between these two cases are that in ours there was less reaction, no giant cells, more hæmorrhage, much less granulation tissue formation, and an extension of the necrotising process beyond the line of infiltration into the structures beyond. In the study of our sections no mast cells were encountered, although plasma cells were abundant. Such differences may readily be due to the variations in the reactive power of different individuals, and to secondary infection and it seems quite probable that the two cases represent the same condition.

III. RÉSUMÉ OF LITERATURE.

There is at the present day some confusion in the differential diagnosis of the various forms of tropical ulceration, and there can be little doubt but that the disease which we treat of in this article has been described at different times under various names. The most complete article dealing with the subject of gangosa which we have found is the recent one by Mink and McLean, and the following summary is taken from this and from the papers of Leys, and of Fordyce and Arnold.

Gangosa was described in 1828 by the Spanish Commission which visited the Ladrone Islands. Similar cases have been reported from the Caroline Islands, Fiji, British Guiana, Italy, Dominica, Nevis and Panama. It probably exists in Ceylon under a different name.[3]

"Gangosa" is a Spanish word and means a "nasal voice." The disease is characterized by a slowly progressing ulceration, starting in the throat, or soft palate, advancing upwards and forwards through the nasal passages, destroying in its progress the septum, hard palate and turbinates, and causing the falling in of the nose. In rather less than 10 per cent of cases the ulceration eventually attacks the anterior nares and leads to more or less complete destruction of the nose, so that it is possible to look through the nose into the mouth and throat. In the later stages the upper lip may be attacked and the process may extend through the lachrymal ducts or across the face to the eyes, leading to secondary inflammations and blindness, the orbit filling up with granulation tissue. Out of eighty-one cases tabulated by Mink and McLean there were only two in whom hearing was affected, while the larynx was involved thirty-three times. In our case there was double *otitis media.* Phonation is always interfered with and the senses of smell and taste are usually lost. It is characteristic that the tongue and muscles of deglutition escape. The ulcers are nearly or quite painless.

In a case reported by Dr. Rat, the ulceration started from a tubercle on the soft palate. Three of Mink and McLean's cases started with mild sore throat or coryza. At first the ulcer is superficial, moveable and covered with a dirty, brownish-gray pelicle. It spreads rapidly, later it becomes chronic and granulation tissue is formed around the base. The ulceration may advance steadily for many years or may become arrested at any time, leaving a chronic ulcer. In most of the cases of Mink and

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[3] On a recent visit to the Batanes Mr. Fergusson, of this Bureau, found several colonies of apparently gangotic sufferers living in quarters more or less isolated from the main villages. These people are regarded as lepers.

McLean the ulceration became quiescent after from one to seven years, the average time being two years, while in seven cases the advance was continuous for from ten to thirty-five years. Periods of advance and quiescence may alternate. During quiescence there is an abundant, very offensive discharge. It is remarkable that the general health is hardly affected, even with extensive and long continued ulceration. An exception to this rule is to be noted in the case of children under 5 years, in gangotic families, who may die within forty-eight hours from a fulminating type of the disease, almost like diphtheria. The ulceration in gangosa is limited to the throat and adjacent parts. No similar ulcers are found in other parts of the body.

We had no opportunity to determine the etiological factors in our case. Mink and McLean analyzed 125 cases at Guam and estimate that about two per thousand of the native population are affected, whereas Arnold gives a higher estimate. No cases have been seen in white people or in those of mixed blood. The majority of all cases develop during the second and third decades, and more during the second than at any other equal interval; but the disease may appear at any age from three to sixty or eighty. Women are attacked more frequently than men. Gangosa is not hereditary, but is probably infectious. The natives think that it comes from eating fish. The degree of infectiousness must be low.

Gangosa must be differentiated from other forms of chronic ulceration. Leprosy can be excluded by the absence of nodules, infiltrations and anæsthesia, the fact that the characteristic bacilli can not be found in the ulcer, by the sudden onset of gangosa, and by the general good condition of the patient. From the other granulomata, the differential diagnosis should not be difficult. Epithelioma may be excluded by the wide prevalence of gangosa in a small community, by the early age of onset, the protracted course, the absence of metastases, the softer base of the ulcer and the histological features.

In certain respects the disease resembles an unchecked Vincent's angina or noma. The protracted course and the absence of general symptoms exclude noma. In our case, tissues stained by Levaditi's silver method, by Gram-Weigert and by the carbol-fuchsin methylene blue method for tubercle bacilli failed to show the spirochæta of Plaut and Vincent.

The differential diagnosis from syphilis and yaws is difficult. We do not wish to enter into the syphilis-yaws controversy at this time, and we will merely state that we have never seen any lesions of yaws which presented the faintest resemblance to gangosa, although the typical framboesial skin lesions are not uncommon in this region. However, it is obvious that gangosa bears a strikingly similar appearance to the ulceration occurring in syphilis, and Hutchinson[4] has presented a strong case for those who maintain that syphilis and yaws are modified forms of the same disease. Neither Mink and McLean, nor Leys, found any syphilis whatever among the natives of Guam where gangosa prevails so widely and they satisfied themselves that there is no casual connection between syphilis and gangosa. Mink and McLean also convinced themselves that the disease is independent of yaws. Fordyce and Arnold excluded syphilis from the diagnosis of their case, because of the long duration of the ulcer in a limited area, the failure of antisyphilitic remedies to relieve the condition, and because they considered it unusual to have the lachrymal duct involved by direct extension of intranasal syphilitic ulceration. In our case, the patient failed to respond to vigorous antisyphilitic treatment. There were no other signs suggestive of syphilis either clinically or at autopsy, the histological features of the ulcer were not suggestive of syphilis and no spirochætæ could be found in the tissues after impregnation with silver according to the method of Levaditi.

Considering both the literature and our case, we think that the weight of evidence is in favor of the view that gangosa is a disease independent of syphilis, but we do not regard this as a definitely established fact.

Tuberculosis must also be differentiated. In our case the patient had unquestionably a chronic pulmonary tuberculosis with a few small tubercles in various organs, but no tuberculous lesion existed along the advancing edge of the ulcer, and no tubercle bacilli could be found in it. In Fordyce's case the inoculation of guinea pigs and the test with tuberculin proved to be negative for tuberculosis, and, as in our case, the general histological appearances were unlike that disease. At the same time, there were giant cells in Fordyce's sections and in both his and ours there were extravasated red blood corpuscles. According to Stelwagon,[5] lupus not infrequently attacks the mucous membranes, and may affect the soft palate, hard palate, or velum, the mucous membrane involvement taking place in 45 or even 70 or 80 per cent of the cases. Against tuberculosis are (1) the painlessness of the lesion, (2) the long duration, (3) the continued good health of the patient and (4) the absence of symptoms of tuberculosis elsewhere.

The considerations enumerated above dispose us to take the view that this patient suffered primarily from gangosa, apparently a separate disease, that the tuberculous process was secondary and that he died of a terminal acute broncho-pneumonia.

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[4] Framboesial Syphilis (Yaws and Parangi); Atlas of Clinical Medicine, Surgery and Pathology of the New Sydenham Society. Part I, Fasciculus XIV (1902).

It will require careful obervations on several cases under prolonged treatment to justify a positive expression of opinion as to the part played by syphilis or yaws in the gangotic process.[6]

REFERENCES.

(1) Fordyce and Arnold: *J. of Cutaneous Diseases* (1906), XXIV, 1.
(2) Leys: *J. Trop. Med.* (1906), IX, 47.
(3) Mink and McLean: *J. Am. Med. Ass.* (1906), XLVII, 1166.

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[5] Diseases of the skin: W. B. Saunders, 4th Ed. (1905), 687.

[6] While this article was in press *Stitt* (U. S. Naval Med. Bull. (1907), I, 96) reported a case of gangosa, the first in a white man. The patient, a sailor, had recently come from Guam, where he had exposed himself to gangosa, but he also had a history of syphilis five years before. Antisyphilitic treatment did not check the disease.

ILLUSTRATION.

PLATE I. A photograph showing the deformity of the nose and lip produced by gangosa.

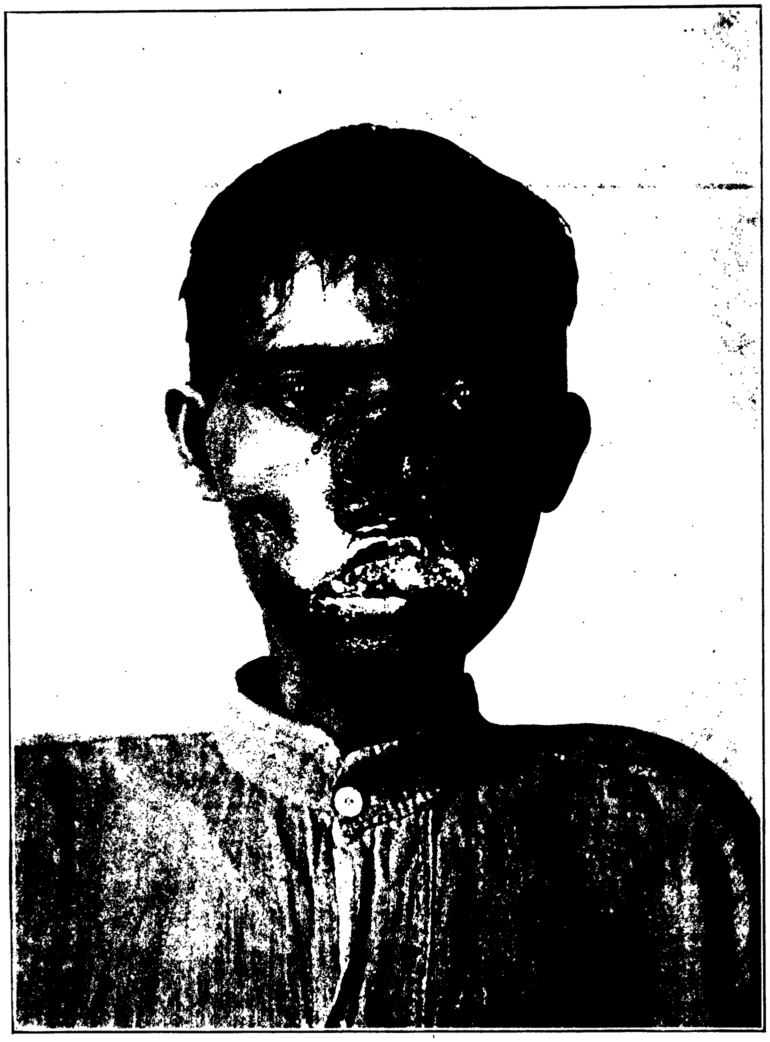

PLATE I.

REVIEWS.

Infection, Immunity and Serum Therapy in Relation to the Infectious Diseases which Attack Man, with Considerations of the Allied Subjects of Agglutination, Precipitation, Hemolysis, etc. By H. T. Ricketts, M. D. Cloth. Pp., x + 600. Price, $2.50. Chicago: American Medical Association Press, 1906.

This excellent review has become so widely known and was received so favorably on its first appearance in the columns of the Journal of the American Medical Association that an extensive analysis seems superfluous.

The book is divided into two parts, a general and a special. In the first 234 pages the facts and theories of infection and immunity, together with a consideration of antibody formation in general, are set forth clearly, interestingly and with reasonable fullness. In this place hæmolysis and allied processes are considered.

In the second division of the book the diseases of an infectious nature are classified in accordance with the theoretical considerations of part one. A review of the recent work which tends to throw light upon the nature of the process involved is given for each disease, and an acccount is given of the methods of immunization and the results obtained. In addition, the etiology and pathogenesis is entered into in some instances, notably in the cases of the protozoön infections.

In general the style is so clear that the book will prove intelligible and interesting to medical men who have not had special laboratory training in immunity research. The general arrangement, subdivision and indexing makes the book available for quick reference.

R. P. S.

Diagnostic Methods. By Prof. Dr. Hermann Sahli. Edited with additions, by Francis P. Kinnicutt, M. D., and Nath'l Bowditch Potter, M. D. Authorized translation from the fourth revised German edition. Cloth. Pp., 1008. Price, $6.50 net. Philadelphia and London: W. B. Saunders Company, 1906.

With the additions made by the American editors, this book forms the most exhaustive and complete attempt yet published upon the subject of diagnosis. Every practicable method of obtaining, classifying and properly weighing data looking to a determining diagnosis is considered, including subjective and objective information as well as that secured by laboratory methods and by the use of instruments of precision. The most remarkable feature of the book is its originality, it being largely built up from Professor Sahli's own experience.

The chapter on history taking and general consideration of subjective data is exceedingly satisfactory and complete, including special consideration of the more frequently occurring and striking local manifestations common to more than one disease, such as œdemas, exanthemata, etc.

Physical diagnosis is clearly explained and some of its phenomena interestingly illustrated by diagrams which are intelligible. Blood pressure and the pulse, including venous pulsation, are given with a clearness which can only be obtained by one thoroughly familiar with the subject and in a manner so attractive as to stimulate one to more careful observation in these subjects.

Stomach disorders have probably received the author's closest attention for many years, and the section of his book dealing with this subject is an interesting and very complete statement of the known facts bearing upon the diagnosis of these complex diseases.

There are a few criticisms upon the book, some of which may be mentioned. The arrangement of the subject-matter is apparently without a well-defined system and is somewhat confusing to the American student; trypanosomiasis is not mentioned and clinical bacteriology is placed here and there in the book and is not considered with the fullness its importance deserves in a work of such magnitude.

The book may be highly recommended.

W. E. M.

The Practitioner's Medical Dictionary. An illustrated dictionary of medicine and allied subjects, including all the words and phrases generally used in medicine, with their proper pronunciation, derivation, and definition. Based on recent medical literature. By George M. Gould, A. M., M. D. Illustrated. Flexible leather. Pp., xvi + 1043. Price, $5.00 net. Philadelphia: P. Blakiston's Son & Co., 1907.

We have put this book to a practical test and have found it admirably to fulfill the object for which it was designed. The practitioner who desires to be abreast of the times can not afford to ignore the beauty and advantages of this new work. The specialist will find it not only an indispensable aid, but he will rarely need to look further than its pages for anything that can reasonably be expected of a medical dictionary. No pains have been spared by author or publisher to make of it a convenient, reliable and complete working guide to the spelling, pronunciation, derivation and definition of all the words and phrases generally used in medicine. Where illustrations could be added with advantage they have been inserted with due regard to economy of space. All new words of importance have been added while retaining all old ones, except those which have deservedly fallen into disuse. Due recognition has been given the metric system and the additions and changes of the new United States Pharmacopœia have been included. The volume contains 1,043 octavo pages, is printed on thin paper of fine quality and will be sure to please and help the student, physician or specialist.

E. C. S.

The Physician's Visiting List (Lindsay & Blakiston's) for 1907. Fifty-sixth year. Limp leather. Price, $1.00. Philadelphia: P. Blakiston's & Co.

This well-known record system maintains the excellence of previous editions. The arrangement of subject-matter is very simple, compact and complete. The information given is limited to essentials given in a brief but satisfactory manner. The Visiting List may be recommended as a time saver if indeed it is not a necessity.

W. E. M.

Retinoscopy (or Shadow Test) in the Determination of Refraction at one Meter Distance, with the Plane Mirror. By James Thorington, A. M., M. D. Fifth edition, revised. Fifty-four illustrations, ten of which are colored. Cloth. Pp., XIV + 67. Price, $1.00 net. Philadelphia: P. Blakiston's Son & Co., 1906.

A clear, concise, condensation of the best that is known of the subject. The author has shown that the subject, which is looked upon as complicated by many, is indeed a simple matter which any physician can understand, and by following the instructions learn the practical technique of making objective refractions. As the writer of this review has been refacting objectively for the past number of years with more than moderate success, without having seen a practical demonstration and having had no instruction in the method other than by following Thorington's method, he feels that his own experience justifies the extolling of the art with which the subject is presented.

The passing over, with but few remarks, of the use of the concave mirror is to be commended, as the treating of it fully in a book of this kind tends to confuse the student.

A typographical error is found on page 40, which is liable to cause considerable confusion to the beginner, especially to one who is not accustomed to handle the plus and minus signs.

The manner described of working out the refraction of an eye when the scissors movement is found, is very simple and most satisfactory.

The axonometer does not appeal to the writer, because when accessories are used, the entire number, including an accurate device for ascertaining the axis of the astigmatism is found in the Geneva Combined Opthalmoscope and Retinoscope, which is much easier of manipulation than the different accessories separately.

J. L. S.

The Practice of Obstetrics, Designed for the Use of Students and Practitioners of Medicine. By J. Clifton Edgar. With 1,279 illustrations, including five colored plates and 38 figures printed in colors. Third edition, revised. Cloth. Pp., 1071. Price, $6.00. Philadelphia: P. Blakiston's Son & Co., 1907.

If the measure of success of an individual's work be attainment of aim, we may congratulate the author of this book, who expresses the desire "to present the subject of midwifery from a practical and clinical standpoint, so that it will best facilitate the requirements of the student of medicine, and of the active obstetrician."

The book is divided into ten parts, and each part carefully and fully subdivided for convenience of reference, which is certainly obtained.

The sections on Hygiene of the Sexual Function, Hygiene of Pregnancy and the Hygiene of the Newly Born are full of good suggestions. In the section on physiological pregnancy, much space is given to the "Examination of Pregnancy" and the possibilities of Rœntgen-ray pelvimetry hinted at. The section on pathological pregnancy is important, containing lengthy discussions on all possible anomalies and diseases of the membranes, placenta and cord, while unusual length of description is given to deformities and monstrosities of the fœtus.

In antenatal diseases of the fœtus, the author regards variola, cholera, typhoid fever and syphilis as being most uniformly harmful to fœtal life. The toxæmia of pregnancy is defined as a state of the blood and of metabolism possibly arising from hepatic insufficiency and is discussed at length under the diagnoses of hyperemesis, eclampsia, polyneuritis, etc.

Abortion and ectopic gestation each have lengthy chapters devoted to them.

The sections on labor, both physiological and pathological, contain very plain, clear statements and illustrations so that it would seem impossible for anyone to go astray after having read these chapters.

Dystocia is discussed from every possible standpoint, even to the adoption of an "anti-dystocia diet" according to Prochownik's scheme.

The section on the physiological puerperium is particularly useful in its clear statements of good management through the period.

Morbidity in the puerperium is careful analyzed and classified. The author does not seem to favor the use of antistreptococcic serum, either as a preventive or as a curative agent in the treatment of puerperal infection.

The section on the "Newly-Born" is very complete and lucid.

The final section on "Obstetric Surgery" includes an interesting study on "Posture in Obstetrics and its relation to increase or decrease of pelvic diameters, also valuable hints as to corrections of faulty positions, the use of the forceps, Cæsarian section, etc."

The size and weight of the book are against it, and there are many repetitions, perhaps unavoidable according to its plan, but the evidences of years of rich clinical experience and diligent research, combined with the art of the teacher in presenting a subject clearly and fully, overcome any slight objections.

The type is good, the illustrations clear and profuse, while the table of contents and index are all that can be desired in their line.

E. J. P.

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B. MEDICAL SCIENCES

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SOME REMARKS CONCERNING KUBISAGARI OR VERTIGE PARALYSANT.[1]

By KINNOSUKE MIURA.
(*Professor of internal medicine in the Imperial Japanese University at Tokyo.*)

An interesting disease has prevailed for a considerable period of time among the working peasant class of Japan, in the Northern Provinces, especially in the neighborhood of Homori and Iwateken. The principal symptoms consist in a dimness of vision, ptosis, diplopia; difficulty in speech, in deglutition and in mastication, as well as weakness in the muscles of the back of the neck and of the extremities. At a later time the investigations of my assistant, Takemura, demonstrated that this same disease occurs in some villages of Tokushimaken on the Island of Shikoku, but in a less pronounced form. My investigations in the regions mentioned above, undertaken in the years 1894 and 1895, demonstrated the affection to be identical with endemic paralytic vertigo observed by Gerlier in Ferney, Switzerland. So far as is known to me, no similar reports have come from other parts of the world and therefore I have called your attention to this disease.

Endemic paralytic vertigo (or kubisagari) has, if I can compare it with a disease known to you, a great similarity to myasthenia gravis, with this difference, that our disease appears more generally in the summer time in restricted neighborhoods where it is endemic and epidemic, and that the paresis appears simultaneously in various muscular regions. The symptoms are as follows:

1. Eye symptoms, namely, ptosis, dimness of vision, diplopia, hyperæmia of the papilla and its surroundings.
2. Motor disturbances of the tongue, and lips and interference with mastication, more seldom with deglutition.
3. Paresis of the muscles of the back of the neck and gluteal region.

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[1] Read at the Fourth Annual Meeting of the Philippine Islands Medical Association, Manila.

4. Paresis of the muscles of the extremities.

According to Gerlier there may also be added neuralgic radiating pains to the back of the neck, back and to the periphery (such as forehead, shoulder and arms); more seldom an increase of the secretions of the nose, tear ducts and salivary glands is observed. Cramps, anæsthesia, hyperæsthesia, and disturbances of the organs of sense, or of the bladder and large intestine are always lacking.

To begin with, the symptoms observed in the eyes, obscureness of vision and ptosis, are the most important. The first of these begins with a blurring of the contours of objects which are observed; the patients state that they see everything as if it were surrounded by a fog. Some remark that they see near objects indistinctly, with others it is the reverse, still others have impaired vision both for near and far objects. In addition to the dimness of vision, ptosis is a frequent symptom and one which is more easily shown in an objective manner. This can exist in a slight degree even at times when the patient is otherwise apparently free from the disease. It appears in different degrees; it may be so slight that it only gives the patient a sleepy appearance or it may be so pronounced that only a hair-like slit is seen between the lids. It is brought about by a paresis of the levator muscles of the upper lid and is generally bilateral. Diplopia is less frequent and according to my observation is caused by a paresis of the rectus internus muscle. Gerlier, in addition, mentions photophobia, photopsia and disturbances in the color sense. Eperon, Sulzer and I demonstrated hyperæmia of the papilla, the optic nerves and their surroundings, during the attack.

Disturbances of speech, of the movements of mastication and deglutition are only seen in severe cases. During lengthy conversation the speech gradually becomes more and more indistinct and difficult to understand, because of increasing weakness of the tongue and lips. The muscles participating in mastication and deglutition at the same time lose their normal strength, so that the patient soon is unable strongly to bite on a finger thrust into his mouth, and finally he is unable even to swallow water. It is therefore not a rare occurrence that a person suffering from *kubisagari* loses all his food from his mouth during a meal, instead of swallowing it.

The paresis of the muscles of the back of the neck, back and extremities is observed most frequently when the peasants are working in swampy rice fields bent over their work, as occurs in planting rice and in weeding. Under these circumstances they first observe pressure and heaviness in the back of the neck, the head sinks forward and can only be raised with difficulty, so that in these cases a bandage is tied over the forehead and fastened by a string to the belt, in order to allow the sick one to work. The paresis of the muscles of the loins is manifested by the fact that the sufferer finds difficulty in raising himself to an erect posture after bending forward, and he must support himself by this hands on his thighs or hips;

he also complains of weakness in the body. Paresis of the extremities is manifested by dropping objects from the hands, or the patient moves painfully with a cane or along a wall or similar support. As the ptosis and dimness of vision is in these cases generally intense, the patients are usually compelled to keep quiet and await the passing away of the attack. All the motor disturbances first appear in muscles which are exerted, especially in cases of repeated, identical movements, as for example, during mowing with a scythe, pumping, marching, chewing, writing, etc.

The symptoms given above do not appear simultaneously nor with equal intensity in all cases, but one more generally finds them together in severe attacks; in the lighter ones there is either only ptosis with dimness of vision, or in addition only weakness of the muscles of the back of the neck.

The length of the attack is generally short, it rarely exceeds ten or fifteen minutes, but it reappears readily as soon as the customary work is continued.

The patients are well in the intervals between the paroxysms. They are neither anæmic nor nervous; the liver, spleen and other organs show no abnormalities, the blood contains no parasites either during the attacks or in the interval, the corpuscular elements are also not materially altered. Only patients who have suffered from repeated, severe attacks are left with a certain degree of ptosis and weakness of the muscles of the back of the neck, trembling of the hands, uncertainty of gait and speech (Gerlier) as well as increased reflexes.

Causative factors of the attacks are bodily exertion, notably labor in a stooping position, and repeated, uniform motions, especially on an empty stomach or after the ingestion of food difficult of digestion. Writing, reading, steady attention, mixing with crowds of persons and similar circumstances also act in a similar manner. On the other hand, the attacks are diminished or stopped by rest and change of location. This fact renders the study of the disease in hospitals almost impossible.

In regard to differential diagnosis, in the first place myasthenic paralysis must be taken into consideration as in this disease also, generally by repeated movements, the muscular strength gradually diminishes. On the other hand, in kubisagari there is sometimes a weak indication of the myasthenic reaction, but kubisagari is distinguished from the above-named disease by its endemic-epidemic character as well as by its sudden appearance, by the dimness of vision and by the rapid and more general extent of muscular weakness, etc. It is scarcely necessary here to enter upon a more exact discussion of the differential diagnosis between the disease and neurasthenia (ordinary paroxysmal lameness.)

The etiology of this peculiar disease is obscure. The superstitious peasants in Japan have thought of a supernatural influence of the wandering spirits of the dead who have found no resting place. The Swiss peasants believed in witchcraft. Ladame contends that the epidemic spreading of the infection may be attributed to obscure psychic influences. David suggested adulterated alcoholic beverages, or a poisoning by bread or lentils. Other physicians in Japan and in Europe believe that latent malaria is the cause, but the clinical picture and the blood examination are against this view. Gerlier was the first who emphasized the close connection between this disease and those who work in horse's and cow's stables. After he had excluded the possible etiologic factors, one by one, he emphasized that it appears most frequently in peasants, day laborers, or persons who are occupied with the care of cows and horses, whereas landowners and women are free from endemic paralytic vertigo. Kubisagari also is general in those regions of Japan where a portion of the dwelling is used as a stable for horses or cows and when there is no idea of cleanliness. If we compare the region in Switzerland, where endemic paralytic vertigo is frequently encountered, with Aomori and Iwate, where kubisagari is endemic, we observe great differences in the geological structure of the region and the food of the peasants. Only one point is in common—in Switzerland the custom of sleeping in the stable and in Japan the imperfect separation of stable and dwelling.

Gerlier maintains "dans le bassin de Léman, il est d'usàge qu'on couche á l'etable, ce qui n'est pas admis dans les cantons de Berne et de Tribourg ou la maladie est inconnue." With us in Japan the region of Aomori and Iwate is the territory where agriculture and the raising of cattle take place side by side, so that in each peasant's cottage, horses or cattle are maintained and cared for more carefully than the children. The inhabitants of this region generally have the barn so arranged that a part is used as a stable, and only an incomplete partition exists between the two spaces, so that not only the air but also insects have free access to all parts. The structure of the houses is planned for the winter months. The stable, however, is the place where throughout the year, processes of decomposition are going on and where there is a certain degree of heat in conjunction with imperfect lighting, a true incubator of micro-organisms, for the manure is only cleaned out twice a year.

At this point our knowledge ends. Is the etiologic factor an animal or a vegetable micro-parasite? Is the disease transferred by air, food, insects or domestic animals? The answer to these questions must be left to the future. Horses and cattle seem to resist this disease, but cats and chickens have been observed to be attacked by it, but much more rarely than human beings.

Prophylactic measures are the removal from the neighborhood of horse's or cow's stables and the avoidance of such places for the midday or night sleep. No great hope can be entertained from the use of medicaments, the best results are obtained from a combination of potassium iodide and arsenic. So far as is now known, no deaths have resulted from this disease, although it runs a course with bulbar appearances.

THE INVESTIGATIONS CARRIED ON BY THE BIOLOGICAL LABORATORY IN RELATION TO THE SUPPRESSION OF THE RECENT CHOLERA OUTBREAK IN MANILA.

By Richard P. Strong.

(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

The epidemic of Asiatic cholera which has recently passed through these Islands and has now subsided, occasions me at this time briefly to summarize the work of this laboratory in connection with the suppression of the outbreak. In all, there were 7,085 cases of cholera with 5,243 deaths reported by the Bureau of Health for the Philippine Islands. Dr. Victor G. Heiser,[1] Director of Health, in an admirable article, has recently discussed the origin and history of the outbreak and the general hygienic measures employed in combating the disease. My remarks will be limited to the laboratory measures carried on in connection with the epidemic.

The first case of cholera discovered in the outbreak occurred in Bilibid Prison on August 23, 1905. An autopsy was performed and a bacteriological diagnosis of cholera was reported sixteen hours later to the Bureau of Health. The Philippine Islands had supposedly been entirely free from cholera for the preceding seventeen months. Following the laboratory diagnosis of the first case, others suspicious of this disease were discovered by the representatives of the Bureau of Health, and within the next few weeks a positive bacteriologic diagnosis of cholera had been rendered by the laboratory in over one hundred instances. Soon after the report of the first case was made public, numerous specimens of the fæces of other patients suspected of having cholera were sent to the laboratory for bacteriological study and throughout the course of the epidemic examinations were carried on by members of the laboratory staff either in the central building or in the several hospitals where the suspected cases had been brought. An assistant of this laboratory was also stationed at the cholera hospital and was prepared at any hour of the day or night to undertake the bacteriological diagnosis of the cases admitted. The cholera spirillum was found present in 412 of 582 specimens of fæces examined, and in 304 autopsies performed by members of the laboratory staff on cases supposed to have died of cholera, the diagnosis of this disease was confirmed in 260; 129 specimens of drinking water, collected from reservoirs, wells and other sources of supply were also sent to be examined for infection with the cholera spirillum, but from only 3 of these was this organism isolated.

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[1] *Am. Med.* (1907), **48**, 856.

METHODS EMPLOYED IN THE BACTERIOLOGIC DIAGNOSIS.

Several methods were employed in performing the bacteriologic diagnosis from the fæces, an attempt being made in each instance to secure as prompt a result as possible. All methods which were not based upon the isolation of a pure culture of the cholera organism to be employed in the subsequent tests proved at times to be untrustworthy. The one which was demonstrated to be perfectly reliable in practically all acute cases and by means of which, in addition, a definite diagnosis could usually be reached within six to eight hours and almost invariably in from sixteen to eighteen hours, was as follows: Numerous alkaline agar plate cultures were prepared directly from the cholera stools, some being inoculated with large and others with small portions of the fæces, various dilutions being prepared; the cultures were placed at 37° C. and as soon as the colonies became sufficiently developed, those which resembled colonies of the cholera spirillum were suspended in saline solution. Agglutinative and bacteriolytic tests by the microscopic method were then performed with them and a standard *fresh* cholera serum in proper dilutions. The morphology and motility of the organism were also noted. Frequently, after twelve to sixteen hours from the time of the inoculation of the plates, sufficient growth was obtained in addition to carry out the Pfeiffer bacteriolytic test with the same cholera serum, in the abdominal cavity of a guinea pig. This method frequently, although not invariably, proved to be the quickest means by which a diagnosis could be made, and the preparation of the plate cultures from the suspected fæcal material soon became a routine one in the laboratory. In case a positive diagnosis was reached by methods requiring a briefer period of time, the subsequent agglutinative and bacteriolytic tests by the first described method were not always performed, but if a negative result was obtained by the briefer procedures, then these tests were carried out and they sometimes finally resulted in establishing a diagnosis of cholera.

Another method which frequently could be relied upon for diagnosis in case of a positive result, consisted in making the inoculations from the stool directly into tubes of 2 per cent peptone solution and of the performance of the agglutinative test by the microscopic method with drops of this medium taken from the surface and added to a cholera immune serum. This test, together with the examination of the morphology and motility of the organism, was performed five or six hours after the inoculation of the cultures and in case a negative result was obtained, was repeated after from sixteen to twenty-four hours. Only when positive reactions are encountered by this method can the diagnosis be considered to be conclusive. In case of a negative result, a study of the plate cultures which should previously have been prepared should be resorted to. In some cases in which no agglutination of the organisms which has been cultivated in this way by the enriching process resulted, cholera spirilla were later isolated and identified by means of plate cultures. The success of the peptone solution method in securing a positive diagnosis obviously depends chiefly upon the number of cholera spirilla which exist in the stool.

A number of experiments in diagnosis were also carried on according to the method advised by Dunbar [2] and by means of which an immediate diagnosis of cholera may occasionally be made from the fæces. However, it is worth while to undertake this method only with specimens of excreta in which numerous organisms with more or less typical morphology of the cholera spirillum are present. In stools of this nature the reaction should always be attempted because of the immediate results which may sometimes be obtained. Care must be taken to distinguish pseudo-reactions, and only those cases should be considered as conclusive in which the agglutination is distinct and well marked. The reaction frequently failed in instances of undoubted cholera from which pure cultures of the cholera spirillum were later isolated and identified. The so-called cholera-red reaction, performed with peptone cultures and with nitrite free sulphuric acid, could only be considered in determining the diagnosis when a positive result was obtained, and even then the reaction could only be regarded as confirmatory from a bacteriologic standpoint. A single negative reaction, even though a satisfactory peptone media had been employed, could not be looked upon as an important argument against a positive diagnosis of the cholera organism, since different strains were found to vary in this respect. Obviously, the cholera-red reaction, unless performed with pure cultures of the spirillum, is entirely untrustworthy and the results can not be depended upon even as an aid in diagnosis.

No experiments were performed with the specimens sent for diagnosis, with the object of differentiating by means of the blood-agar of Prausnitz [3] the cholera spirillum from other cholera-like vibrous in the stools, the agglutinative and bacteriolytic tests having by practical experience proved to be satisfactory for clinical diagnostic purposes, notwithstanding the experiments of Kraus. The bacteriologic diagnosis in cases of cholera in the late stages of the disease was sometimes very difficult, and a careful study of the fæces by plate cultures, prepared both directly from the stool and from the surface of peptone solution cultures previously inoculated with large amounts (several cubic centimeters) of the stool, is sometimes necessary in such cases before the organism is isolated. Frequently, an examination of the agglutinative and bactericidal reaction of the blood serum of the patient in these subacute cases will render further assistance in reaching a diagnosis, particularly if the reactions are positive.

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[2] *Berl. Klin. Wchnsch.* (1905), **42,** 1237.

[3] *Berl. Klin. Wchnsch.* (1905), **42,** 561.

PREPARATION AND FURNISHING OF CHOLERA IMMUNE SERA FOR DIAGNOSIS.

In addition to the preparation of fresh cholera immune sera for diagnostic purposes in the central laboratory, such sera were also prepared and furnished to various physicians and institutions in the city and in certain of the provinces for use in the bacteriologic diagnosis of the disease. No dried cholera serum was issued, it having been found practicable and more desirable to furnish a fresh serum by means of which not only an agglutinative test, but also a bacteriolytic one under the microscope according to the method of Bordet could be performed. It was found that a single intravenous inoculation of a rabbit with the immunizing substances extracted from about 60 to 70 milligrams of a virulent cholera organism would furnish a serum of sufficient value for all practical purposes in diagnosis. The animal may be bled to death on the sixth or seventh day after such an inoculation and the serum separated. Such sera almost invariably show an agglutinative value of from 1:1,000 to 1:2,000; values as low as 1:800 have been obtained only in exceptional cases. The bactericidal almost invariably exceeds the agglutinative value of the serum prepared in this manner, the different sera reacting bactericidally in amounts of from 0.2 to 0.05 milligram.

After the serum had been standardized it was sealed in test tubes and was ready for delivery. Fresh serum produced by this method was kept on hand throughout the epidemic.

PROPHYLACTIC INOCULATIONS.

The laboratory in addition to the diagnostic work was particularly occupied in the preparation and standardization of cholera prophylactic and in the performance of the protective inoculations against the disease in certain badly infected cholera districts in Manila and the provinces.

The prophylactic prepared and employed consisted of the immunizing substances extracted from the cholera spirillum and suspended in saline solution.[4] The method of its preparation has been described in detail in former articles from this laboratory. It will be sufficient to state here that an organism known to possess high immunizing and peptonizing powers and one of maximum virulence is selected and grown upon the surface of large test tubes containing 1 per cent alkaline agar. After twenty hours, the growth of one-half of the number of inoculated culture tubes is suspended in 0.85 per cent saline solution, 1 cubic centimeter being employed for approximately every 30 to 35 milligrams of bacteria. The suspension is heated for one hour at 60° C. and then placed at a temperature of 37° for from three to four days. At the end of this time its sterility is tested, it is filtered through a Berkefeld candle and the filtrate saved. The remaining half of the twenty-hour agar cultures is suspended in sterile distilled water, 1 cubic centimeter to each 30 to 35 milligrams. This suspension of the *living* organisms is then placed on an electrical shaking machine and throughly shaken for from three to four days. At the end of this time cultures are taken to ascertain if the growth is pure and the suspension is then also filtered through a Berkefeld candle. The two filtrates are subsequently mixed in equal proportions and carbolic acid added to 0.5 per cent. The prophylactic is finally bottled in glass flasks, the smaller ones being sealed in the flame. Two cubic centimeters of this mixture represents an adult dose. After the sterility of each sample of the vaccine has been tested by animal inoculation and by anaërobic cultures, it is standardized according to the number of units of immunity to which it gives rise after inoculation; one unit of immunity equaling the amount of immune serum which will protect a guinea pig of 250 grams weight against the intraperitoneal inoculation of ten times the fatal dose of living cholera organisms. If 1 cubic centimeter of the vaccine, when injected intravenously into a rabbit, does not give rise to at least 10,000 units of immunity, it is not issued for human inoculation. No method of standardizing the small amount of anti-endotoxin which the prophylactic will give rise to in animals has been found to be either practicable or of value. During the period of the epidemic the prophylactic was manufactured and used in large quantities and a sufficient supply was kept on hand for emergencies.

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[4] The process by means of which this prophylactic is prepared may be regarded as the outcome of the experimental work performed by Koch, Neisser and Shiga, Wassermann, Brieger, and myself. The idea of using the immunizing substances (free receptors) extracted from *Bacillus typhosus* and *Bacillus dysenteriae* for producing immune sera originated in Ehrlich's laboratory with Neisser and Shiga[5] in 1903, and that of using the extracted free receptors of the cholera spirillum for prophylaxis against cholera in Wassermann's laboratory a few months later (during the same year) where I[6] was able to carry on the first extensive and conclusive experiments in regard to its value. Later Shiga[7] advocated and used the method for preparing a prophylactic against typhoid fever.

Brieger and Mayer[8] called attention to the advantage of extracting the soluble substances of the organisms by shaking in distilled water.

[5] *Deutsche med. Wchnsch.* (1903), 4, 61.

[6] *Am. Med.* (1903), **6**, 272.

[7] *Berl. klin. Wchnsch.* (1904), 4, 79.

[8] *Deutsche Med. Wchnsch.* (1904), **30**, 980.

EFFICIENCY OF THE PROPHYLACTIC.

I called attention to the fact in my previous publications on the subject of protective inoculation against Asiatic cholera, that animals could invariably be protected against a subsequent cholera infection with even multiple lethal doses of the organism as the result of a single inoculation of this prophylactic, in doses of from 1 to 5 cubic centimeters: and that in addition agglutinative and bactericidal substances became developed in considerable quantities in the blood sera of such animals. However, the antitoxic value of these sera was only moderate. It was also demonstrated that the antibodies which developed in the blood of individuals after inoculation with the prophylactic were identical with those which were encountered in patients convalescent from cholera, and, in addition, that these immunizing substances were frequently present in greater amounts after vaccination than after a natural attack of the disease. Moreover, it was shown that animals which contained these same antibodies in sufficient quantities in their sera were also invariably immune to cholera infection, the amount of the immune bodies in the serum being proportional to the degree of immunity to infection possessed by the animal.

From a single intravenous inoculation of rabbits with an amount of the prophylactic containing the receptors extracted from 2 oesen of a virulent cholera organism, sera were obtained which showed an agglutinative value of from 1:300 to 1:600, and a bactericidal one of from 0.1 to 0.08 milligram, while from a similar inoculation of the receptors extracted from 12 oesen of the same strain, sera resulted showing agglutinative values of from 1:600 to 1:1,000 and bactericidal values of 0.08 to 0.04 milligram. From a single intravenous inoculation of ½ oese of a *living* agar culture of this same cholera strain, sera were obtained with an agglunative value from 1:400 to 1:800 and a bactericidal one of from about 0.1 to 0.06 milligram.

It is important to observe that 0.5 oese of the living organism gave rise to sera of almost the same value as did the receptors which could be extracted from 2 oesen of the bacteria, but that the receptors extracted from 12 oesen of the organism furnished sera of far greater value. The best antitoxic serum which was produced following a single intravenous inoculation of the prophylactic was found not to be able to neutralize above four lethal doses of the cholera endotoxin.

From the subcutaneous inoculation of rabbits with 5 cubic centimeters of this prophylactic containing the receptors which could be extracted from 40 oesen of the same strain, sera were obtained with an agglutinative value of from 1 : 500 to 1 : 600 and a bactericidal one in from 0.14 to 0.1 of a milligram. Animals have been found to retain these immune bodies in their sera for as long a period as one year.

A brown powder was obtained by evaporating the prophylactic to dryness in a vacuum. This was placed in sealed tubes and when desired for use redissolved in saline solution. The intravenous inoculation of rabbits with from 3 to 10 milligrams of this redissolved powder furnished sera of an agglunative value of from 1 : 50 to 1 : 100 and a bactericidal one of from 2.5 to 0.25 milligram. These sera were obviously of much lower value than were those which resulted from the inoculation of the prophylactic before evaporation.

In man after the subcutaneous injection of 2 cubic centimeters of the prophylactic, sera showing agglutinative values of from 1 : 40 to 1 : 600 and bactericidal ones of from 1 to 0.25 milligram were obtained.

The advantages which this prophylactic seems to possess over other forms of anti-cholera inoculation are:

First, there is practically no local reaction or only a slight one after its use, the irritating oxidizing substances which existed in the bodies of the bacteria and which have nothing to do with the immunizing substances, having been removed.

Second, we therefore are able to inject an amount of these immunizing substances which is from fifteen to thirty times as great as would be practicable if either the living or killed bacteria were inoculated. Both Haffkine and Murata in their extensive inoculations in human beings injected from 2 to 4 milligrams of culture. In our human inoculations the immunizing substances extracted from about 60 to 70 milligrams of culture are inoculated, hence a higher immunity is obtained by this procedure.

Third, the prophylactic may be sealed in flasks and stored ready for use and it preserves its immunizing properties for at least a year.

The great disadvantage which the method possesses is that each step of the manufacture of the prophylactic must be carried on with great care. It is obvious that the product which we recommend and employ is far more difficult to prepare than is either a simple suspension of the killed or of the living cholera organism. A well-equipped laboratory and trained assistants are necessary for its manufacture. However, it is equally clear that a higher immunity against cholera infection can be obtained by a single injection of this prophylactic than by single inoculations of either the killed or living organisms. The reasons for this have already been emphasized.

Therefore, it has been shown that it is easy to produce in man a high bactericidal serum by the injection of the prophylactic just described. However, the question arises whether such a serum in man really represents an immunity against the disease Asiatic cholera—that is, does it protect the individual against intestinal infection? This question apparently resolves itself chiefly into one as to whether the organisms which give rise to the symptoms of the disease actually come into contact with the fluids of the body.

All articles upon the pathologic histology of cholera agree that the spirilla are found in the superficial layers of epithelial cells of the mucosa, and, sometimes in large numbers, penetrate well into the submucous lining. Exfoliation of the epithelium is almost a constant occurrence in severe infections, and in cases of longer standing the organisms are sometimes encountered in large numbers at the bases of erosions or ulcerations which have resulted in the large intestine. In addition, the mucosa is usually distinctly œdematous, and there obviously is no doubt but that the bacteria in any of these situations would come into contact with whatever immunizing substances might exist in the blood serum, and if bactericidal substances were present in sufficient strength, the bacteria would be destroyed. However, we know that very large numbers of the cholera spirilla remain in the lumen of the intestine in the rice-water discharges and do not invade the intestinal coats. We do not know whether these organisms which so remain in the bowel give rise to the symptoms of the disease, since satisfactory evidence that the cholera spirillum produces a soluble toxin has not yet been presented; but even granting for the moment that they do, then what influence if any would bactericidal substances in the blood serum exert upon them? We are aware that one of the most common and striking findings at post-mortem examinations in Asiatic cholera is the so-called "rice-water" contents of the small intestine. The characteristics of the rice-water stool are due to the flakes of mucus and the epithelial cells which appear in suspension in the liquid contents. If we examine these floccules of mucus bacteriologically we find that the spirilla are usually most abundant in them, sometimes in almost pure culture. However, this mucus is largely a secretion of the epithelial cells of the intestine, although it is usually also mixed with a certain amount of serum and such a secretion will probably possess the same bactericidal substances as the blood serum itself, just as the sweat, the tears, and the milk of immunized individuals contain these antibodies. Indeed, it seems very probable that the intestinal epithelial cells which give rise to the mucus are perhaps particularly able to produce these immunizing substances, since there is probably a special affinity or combining power between these cells and the cholera organism, which is demonstrated by the production of toxic substances in far greater amount in the human intestine than the injection of these bacteria into the circulation, or beneath the skin either of man or animals ever gives rise to.

Having investigated the agglutinating power of a fresh extract obtained from the intestinal epithelial cells of two rabbits previously inoculated intravenously with the immunizing substances extracted from the cholera organism by autolysis, and having found traces of agglutinins present in these extracts, it occurred to me that it would be advisable to undertake the investigation of the bactericidal value of the rice-water stools obtained from cholera cases and also to examine whether the cells of the intestinal mucosa in rabbits immunized with our cholera prophylactic possessed an increased affinity for cholera receptors. It was also of interest to discover if extracts of these cells from the inoculated rabbits possessed bactericidal properties. This study was undertaken by Dr. Edwards of this laboratory who reported his results at the Third Annual Meeting of the Philippine Islands Medical Association in 1906. Owing to the difficulties encountered in the technique, produced by decomposition of the extracts obtained from the intestinal cells, admixture with other bacteria, etc., he was not able to arrive at any satisfactory conclusions in regard to these questions.

It has been supposed that when cholera organisms are injected intravenously into animals, the immunizing substances are anchored particularly to the cells of the spleen, the bone marrow, and lymph glands, since it was in these organs, according to the investigations of Pfeiffer and Marx,[9] that the specific protective substances seemed particularly to be formed. However, Wassermann and Citron [10] demonstrated that the location of the development of the immunizing substances depends to a large extent upon the point at which the injections of the corresponding bacterial antigens were made. Nevertheless, the combining power of intestinal epithelial cells for the receptors of the cholera spirillum has not been definitely determined,[11] although as has been mentioned it seems not unlikely that in the human intestinal infection the epithelial cells of the mucosa may possess receptors with special combining powers for the corresponding cholera antigens and hence give rise to the production of amboceptors (immunizing substances) in excess, which may pass off in the exudates from the cells, as for example in the intestinal secretion containing mucus and serum. However, while it is interesting to review this evidence that the organisms in the intestine in Asiatic cholera do, indeed, come into contact with the serum and tissues of the host, the most definite proof that this really takes place is furnished by the fact that after an attack of the disease, both bactericidal and agglutinative substances are produced and may be demonstrated in the blood serum of the individual. It therefore seems to be proved that if cholera organisms should find their way into the intestine of one whose blood serum possesses a high bactericidal power against the spirillum, the individual in question would be in a much more favorable condition for overcoming the infection, or indeed of throwing it off entirely, than if no such bactericidal substances were present.

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[9] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1898), **27**, 272.

[10] *Ibid.* (1905), **53**, 331.

[11] Brieger and his assistant took *per os* repeatedly from 5 to 15 centimeters of Brieger's vaccine (aqueous extract of typhoid bacilli) but no development of bacteriolysins occurred in their blood.* This experiment throws no light upon the subject since perhaps, the bacterial antigens were changed or destroyed by the gastric and intestinal juices before their immunizing power was exerted upon the intestinal cells.

* Bischoff, H.: Das Typhus-Immunisierungsverfahren nach Brieger. *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1906), **54**, 298.

Haffkine's extensive statistics demonstrate this fact conclusively and show the value of protective inoculation against this disease, the number of cases of cholera among the inoculated being only about one-tenth that observed in the uninoculated. I have no such extensive statistics to offer in regard to the use of our prophylactic, since we have pursued our inoculations during the past year only in those districts where it was thought that the value of the inoculations might be clearly determined. The first town in which extensive vaccinations were practiced was Angat and its barrios in the Province of Bulacan. The village is situated directly upon a small river from which it derives its entire drinking water supply. Considerable drainage from the town in wet weather passes into this river. Cholera was not present in the town at the time the inoculations were performed, but it had been present there a short time before and it was thought probable that it might recur in that locality during the rainy season. About one-sixth of the population of the village—that is, all who volunteered, 1,078 in number—was injected with the prophylactic. A few months later cholera appeared in the village and 122 persons were stricken with the disease, 121 of whom were among the noninoculated. In the villages of Siniloan, Mabitac, and Malolos, 2,706 persons were inoculated, but since the inoculations were performed there has not been sufficient cholera in these localities to draw conclusions of any particular value as to their efficacy. However, only three of the entire number of persons inoculated have contracted the disease. In Bilibid Prison a little over one-half (1,838) of all the inmates was inoculated. During the twelve months following the injections, according to official reports of the Bureau of Health, there have been twenty-four cases of cholera in the prison, only four of which were among the inoculated.

REVIEW OF THE RECENT WORK UPON PROTECTIVE INOCULATIONS AGAINST CHOLERA.

Having briefly outlined the work carried on in this laboratory in connection with prophylaxis against cholera, I wish to review the results which several other observers have obtained with different methods of anti-cholera inoculation, since the publication of my last article upon the subject.[12]

Bertarelli[13] in April, 1905, performed a few experiments in cholera immunization, using for the injections the receptors of the spirillum separated by autolysis, but the strength of the autolytic product employed is not given. He inoculated himself subcutaneously with 3.6 cubic centimeters and a rabbit with 5 cubic centimeters of such a prophylactic and was able for more than six months after the inoculation to demonstrate agglutinative and bactericidal substances in the blood in each instance. During the previous year, I had already shown that in the blood these antibodies persisted for a longer period of time than six months after inoculation with the immunizing substances extracted by autolysis from the organism. Bertarelli's experiments do not appear to be sufficiently extensive to throw much light upon the value of the method of immunization with the autolytic extracts of the cholera organism, although he apparently considers this method of inoculation of value.

Heller,[14] (Schweizer Serum und Impfinstitut, Bern), after pointing out the advantages of protective inoculation against cholera with cholera nucleo-proteid prepared by Lustig's method, reports the results of an experiment in a rabbit in which the animal had finally received 0.25 gram of the nucleo-proteid. The serum was then tested and showed an agglutinative value in a dilution of 1: 400. By finally inoculating 0.8 of a gram of the nucleo-proteid this value was increased to 1: 1,000. A rabbit which was inoculated with the entire cholera organism produced an agglutinative serum in a dilution of from 1: 1,000 to 1: 3,000. The author emphasizes the fact that the injection of the nucleo-proteid causes but a moderate reaction and gives rise to a high immunity which lasts for months, and that the prophylactic does not readily deteriorate.

Friedberger and Moreschi,[15] in a study of the comparative value of the active immunization of rabbits against cholera and typhoid infection obtained by different methods, performed numerous experiments with cultures of the cholera organism killed by diverse means and with others dried at high temperatures or autolytically digested. Their conclusions in this article throw but little light upon the value of autolytic digestion as a practicable means of obtaining the immunizing substances from the spirillum for use in cholera prophylaxis. In their conclusions regarding this subject, they state that with the Pfeiffer-Kolle method, or with one recommended by Loeffler (in which the bacteria are killed at 120° C.), autolysis carried on at body temperature produces no distinct influence upon the activity of the antigens in immunization, and that certainly these substances do not become increased. In another portion of their article they point out that three days' autolysis of cholera cultures killed at 60° causes no loss of their ability to produce bacteriolytic substances upon injection.

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[12] *Publications of the Bureau of Government Laboratories*, Manila (1904), **16**, 1, and *J. Infect. Dis.* (1905), **2**, 107.

[13] *Centrbl. f. Bakteriol. Orig.* (1905), **38**, 584.

[14] *Ibid*, **39**, 106.

[15] *Ibid*, 453.

Obviously, a given cholera organism is endowed with a certain number of receptors. It is difficult to conceive how these receptors could be increased by autolytic digestion of the organism. The advantage of autolytic digestion in obtaining the immunizing substances from the cholera spirillum for use in prophylaxis is that it permits these substances to be separated from other injurious and nonimmunizing substances in the protoplasm of the cell, and hence permits of the inoculation of a larger dose of immune bodies than does the method in which the entire bacterial cell is injected.

A consideration of great practical importance—namely, the influence of the size of the dose upon the antibody production—is discussed by Friedberger and Moreschi, who point out that 1/500 oese injected intravenously gives rise to the production of the same amount of antibodies not only as 1/100 or even 1/10 oese, but to the same amount as does a 2,000 times larger injection, namely 4 oesen. These results of Friedberger and Moreschi have not been confirmed by other authors, and the immunity obtained in cholera must still be considered within certain limits to be proportional to the dose inoculated, as I pointed out several years ago.[16]

Schmitz,[17] in a very exhaustive article from the Institute for the Investigation of Infectious Diseases in Bern, calls attention to the immunizing value of cholera prophylactic prepared according to the method of Lustig, and shows that by its use animals may be immunized against cholera infection and that, following inoculations with it, both agglutinative and bacteriolytic substances develop in their sera. However, these antibodies according to his experiments were not produced in very great amounts, an agglutinative value of only 1: 400 being obtained after an injection of 0.25 milligram of the vaccine and only one 1: 800 after the size of the dose had been increased to 0.8 milligram.

B. Klein[18] performed a few experiments for the purpose of comparing the value of immunization with killed agar and bouillon cultures of the cholera organism with that produced by the autolytic extracts of the spirillum. In all, eleven animals were immunized, four with the autolytic extracts and five with the killed cultures. All were found later to be immune to cholera infection. In concluding his remarks the author quotes Wysokowicz, who states that it is still unproved how long the immunity is retained after inoculation with the autolytic extracts of the cholera organism and that the method of preparation of the prophylactic is more complicated than with that recommended by Kolle. However, in very susceptible persons the autolytic extracts are recommended because of the fact that the local and general reaction following their use is milder than when Kolle's method is employed.

Serkowski[19] during the epidemic at Lodz inoculated eighteen persons, eleven with killed agar cultures of the cholera organism and seven with the separated free receptors. Upon the later examination of the bactericidal properties of the blood serum of the inoculated, he found no difference in value between those vaccinated with the killed cultures and those with the extracts of the organism. However, he points out that the preparation of the vaccine according to the former method is much simpler. The size of the dose employed in either method is not given. In the further inoculation of a number of human beings with killed cultures, in some of whom the injection was repeated a second and third time, it was demonstrated that there was a distinct relationship between the bactericidal immunity obtained and the size of the dose. However, there appeared to be no direct relation between the size of the dose and the agglutinative value of the blood, nor between the agglutinative value and the bactericidal power; neither did the number of vaccinations seem to be directly related to the formation of the agglutinins.

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[16] *Publications Bureau of Government Laboratories, Biological Laboratory* (1904), **21**, 1. *J. Exp. Med.* (1905), **7**, 229.

[17] *Ztschr. f. Hyg. u. Infektionskrankh.* (1905), **52**, 1. *Centrbl. f. Bakteriol. Orig.* (1906), **41**, 118.

[18] *Centrbl. f. Bakteriol. Orig.* (1906), **41**, 118.

[19] *Ibid*, 255.

Meinicke, Jaffé and Flemming[20] have carefully considered the binding power of the cholera vibrio in relation to the production of immunity. Their experiments performed upon the relation between binding power and virulence are of great practical importance in regard to the subject of protective inoculation in man. They conclude that binding power and virulence are independent of each other, since in some cases the avirulent cholera organism revealed a greater binding power and in others a lesser than certain more virulent ones. They believe that the apparent quantitative differences *in the binding power between different cholera strains* can be explained by the qualificative differences in the structure of the receptors of the organism. They also conclude, although their experiments in relation to this point are few in number, that the virulence of a cholera culture bears no relation to its immunizing power. They were unable to confirm the work of Friedberger and Moreschi in regard to obtaining sera of as high a value from the intravenous inoculation of 1/100 oese of a cholera culture as from a much larger dose. Even with the intravenous inoculation of 1/10 oese they were able to produce sera of moderate value only in about half of the animals inoculated. Differences in the value of the sera were much greater when the small doses were used than when larger ones were employed. They believe that Friedberger and Moreschi's results can be explained by the fact that in immunization with such small doses, the value of the serum obtained depends largely upon individual variations in the animals furnishing the serum.

Fichera's,[21] experiments in relation to binding power and virulence are mainly confirmatory of those of Meinicke, Jaffé and Flemming. This author found that strains of the cholera organism which had been isolated for long periods of time still possessed the same binding power for cholera amboceptors as freshly isolated cholera cultures. Fichera also investigated the relation between the immunity produced and the size of the dose. He found, contrary to Friedberger and Moreschi, that rabbits inoculated intravenously with 1/100 of a 24-hour culture killed at 60° C. furnished sera which had an agglutinative value of about 1/10 or even less of that furnished by animals inoculated with 1/20 of the culture. The bactericidal value of the sera obtained from the inoculation of 1/100 of a culture was about one-fifth the value of the latter. The results of Friedberger and Moreschi, as the author points out, may be explained on the ground of individual variation in the immunity of the different animals. A human being was inoculated intravenously with 1/100 oese of a cholera culture killed at 60°, but no practical increase in the immune bodies of the serum was demonstrated, therefore the author does not recommend this small dose for active immunization. Fichera recalls that, with those methods of cholera immunization in which specific sera are added to the bacteria before inoculation, the immunizing value of the organism is lost in proportion to the saturation of its receptors with amboceptors before the injection. In case the vibrios were saturated, so to speak, with the serum, the animals were only immunized slightly or not at all.

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[20] *Ztschr. f. Hyg. u. Infektionskrankh.*, Leipz. (1906), **52**, 416.

[21] *Centrbl. f. Bakteriol. Orig.* (1906), **41**, 576, 671.

Karwacki[22] inoculated nine persons with about 1 oese of killed cholera organisms suspended in saline solution. After five days a second dose of 2 oesen was injected, following which, apparently quite marked local reaction occurred. The reactions were usually milder after the second than after the first injection. After the first vaccination the bactericidal value of the serum was 0.02 in eight of the cases (50 units); after the second the sera showed values of from 2,000 to 10,000 units. The agglutination after the first inoculation in no case reached over 1: 50. In some instances there was no reaction in dilutions of 1: 5, while after the second vaccination the agglutination was in no case below 1: 5 and in one it had reached 1: 400.

Blell[23] (from the Institute for the Investigation of Infectious Diseases in Bern) has also reported in detail upon the value of cholera immunization with cholera nucleo-proteid. The agglutinative and bactericidal value of the blood sera of a large number of animals was studied. Many of these had received repeated and increasing doses of the prophylactic. In two cases the bactericidal value of the rabbit's blood was determined after single injections of 0.1 gram and 0.05 gram, respectively, of the nucleo-proteid and was found to be 5 milligrams and 10 milligrams. In the rabbits which had received a number of repeated inoculations, sera to a maximum value of 0.8 of a milligram were obtained.

The author also reports experiments from the results of which he believes that cholera immune serum produced by inoculation of the nucleo-proteid may exert a curative effect on animals which are inoculated with it in from one to four hours after infection with living cholera spirilla.

Finally, we have the report of Haffkine[24] which gives a summary of the work performed on anti-cholera inoculation in India. Haffkine refers to the recent work of Pfeiffer and Friedberger and of myself, which seemed to demonstrate that the vaccinating power of a cholera culture varies in direct relation with the degree of its virulence, a principle he remarks which served Pasteur for twenty years as a basis for his "*traitement intensif*" in rabies. Haffkine points out that for this reason in the beginning of his work on protective inoculation against cholera he sought to obtain a "*virus fixe*" with the cholera vibrio.

In his very extensive inoculations in man he has observed that the intensity and duration of the symptoms provoked by the subcutaneous inoculation of the living vibrios are directly proportional to the virulence of the culture and the quantity injected. He again describes the methods by means of which the fixed and the attenuated virus used in making the inoculations are prepared. He has found that if the cultures of the organism are killed by heat or by antiseptics such as carbolic or inorganic acids, or by other means, they retain the power of producing an immunity upon inoculation, but this is considerably reduced. The reaction produced after injection of the killed cultures was of the same nature as that brought about by the living ones, only it was less intense. Up to the year 1895 Haffkine always employed two vaccines, the first consisting of a culture attenuated by growing it in contact with the oxygen of the air, and the second, a virus of fixed virulence obtained by passage through guinea pigs. Since 1895, owing to the fact that it was found impracticable sometimes to give the second inoculation, experiments were made by Haffkine and Powell to see if the first vaccination with the attenuated culture could not be omitted. As much as $\frac{1}{6}$ of a gelatin culture of the fixed virus alone was injected in a large number of cases without any serious illness resulting. Between April, 1896 and 1899, somewhat more than 6,500 cases were vaccinated in this manner. Powell, in a report of these cases in 1899, showed that among 6,549 nonvaccinated individuals there were 198 cases and 124 deaths from cholera, while among 5,778 of the vaccinated there were only 27 cases and 14 deaths.

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[22] *Ztschr. f. Hyg. u. Infektionskrankh.*, Leipz. (1906), **54**, 39.

[23] *Ztschr. f. Hyg. u. Infektionskrankh.*, Leipz. (1906), **55**, 187.

[24] *Bull. Inst. Pasteur* (1906), **4**, 694 and 737.

Haffkine points out that while the vaccinated individual is obviously less apt to contract cholera than the nonvaccinated, if the former should actually be striken with the disease he is as likely to succumb to it as the latter (since no marked antitoxic immunity has been produced by the vaccination). The immunity following the vaccination may persist for fourteen months, after which time it diminishes and probably disappears. During the period of active immunity the number of cases of cholera among the vaccinated is but one-tenth of that observed in the uninoculated.

The statistics which Haffkine quotes in his paper conclusively prove the value of protective inoculation against this disease.

We see from this review of the recent work on protective inoculation against cholera that three observers, all from Bern, have reported upon the value of cholera nucleo-proteid as a means of immunization against the disease and have performed numerous experiments showing that antibodies enter into the blood sera of animals inoculated with this form of prophylactic. From single inoculations in rabbits, sera having as high an agglutinative value as 1: 500 and a bactericidal one of 5 milligrams were obtained, but none were higher. However, these values are somewhat low when compared with those I have encountered in rabbits after a single inoculation of the prophylactic I have described.

Turning now to the experiments which have been performed by other observers, it may be seen that no very extensive studies other than my own have been made with the immunizing substances of the cholera spirillum obtained and separated by autolysis, either in man or animals. All of those who have reported upon the use of the method have apparently lost sight of what seems to me to be its most important advantage, namely, that when the immunizing substances are extracted from the cholera organism they may be injected in much larger amounts at one time than if the whole organism is used. I have inoculated myself with three oesen (6 milligrams) of a living, virulent, cholera organism at a single dose, and the local and general reaction experienced was such as to make me conclude that a larger amount than this would not be practicable nor desirable as a method for general inoculation. On the other hand, as I have pointed out, our routine method of human inoculation consists of the injection of the immunizing substances in colloidal suspension or in solution, extracted from 60 to 70 milligrams of the bacteria. Obviously, we are not able to separate and obtain all the receptors of the organism by the method I have employed. This was demostrated by my earlier experiments, when it was shown that ½ oese of the living organisms furnished as good immune sera as the receptors extracted from 2 oesen of the same culture. However, the receptors extracted from 12 oesen gave rise to far better sera. A much larger dose than ½ oese of the living organisms could not have been resisted by the animals, as they would have succumbed to an infection with the spirillum.

LOCAL REACTION FOLLOWING INOCULATION.

Several of the observers who have used in man the method of injecting extracts obtained by autolysis remark upon the fact that the local reaction is less marked than in the case of the inoculation of either the killed or living spirilla. There can be no doubt of this fact and we have had opportunity to compare the reaction produced by each of these methods in man as well as in animals. In animals the differences are very striking and may easily be observed. If a guinea pig is inoculated in the abdominal cavity and tissues overlying the abdominal wall with ½ oese of a virulent cholera organism of which the lethal dose is about 1/10 of an oese, or with 5 or 6 oesen of the same killed organism, at autopsy in the neighborhood of the track of the syringe needle there is found a hæmorrhagic and infiltrated area which is usually sharply circumscribed and of a bright or dark red color. If, on the other hand, a guinea pig is inoculated with a large dose—for example, 5 cubic centimeters—of the extracted prophylactic I have described, the animal may succumb to the injection from intoxication, but at autopsy no hæmorrhagic area will be observed about the point of inoculation. The walls of the abdomen are swollen and œdematous, but there are no evidences of an acute, inflammatory process such as occurs when the living or killed organism itself is inoculated. On the other hand, if inoculations in amounts just below the lethal dose are made with each form of prophylactic, no difference in the quality of the immunity can be detected in the two animals, variation only being found in relation to the *quantity* of the amboceptors; the animals inoculated with a large amount of the prophylactic yielding a better serum than those which had been given the living organisms.

Therefore, it seems unquestionable that there are other irritating substances in the cell of the cholera organism which have nothing to do with the production of the immunity, and it is these substances which may be separated to a large extent from the immunizing antigens by autolysis.

The action of these irritating substances in the cholera cell seems to be particularly active when a large amount of the organism in concentrated suspension is introduced into the tissues; thus, in deaths from Asiatic cholera we do not see markedly hæmorrhagic areas in the intestinal wall where the organisms are spread out over the surface of the mucosa, but if several oesen of the living organisms are introduced into the subcutaneous tissues in man, the hæmorrhagic condition may be produced, as I have demonstrated by an experiment performed upon myself. Inoculation of the human being with the extracted prophylactic which I have described does not produce this hæmorrhagic change in the tissues. Haffkine in relation to this question and of the inoculation of this fixed virus says:

> En inoculation souscutanée au cobaye, le virus ne tuait pas, mais produsait une mortification des tissus et une eschare de grand extension.

If this local reaction is entirely due to the immunizing substances of the cholera organism, why are not similar lesions encountered elsewhere in the animal body? Cholera is an intoxication and it seems almost unreasonable to suppose that all of the toxin is bound locally, either in the intestine in natural infection or in the subcutaneous tissues after artificial subcutaneous injection; the immunizing substances are soluble and must pass to the other organs of the body. It rather appears that with the cholera spirillum, as with the diphtheria bacillus, we have to do also with substances within the bacterial cell which give rise to a local inflammatory reaction, but which have nothing to do with the true immunity in the disease. However, in cholera, of course the toxin is of an entirely different nature from that encountered in diphtheria and probably only becomes liberated at the time of the disintegration of the spirilla.

Before ending this discussion upon the subject of the local reaction produced by the extracted prophylactic, I wish to call attention to the fact that Hetsch and Kutscher,[25] who employed the method of inoculation of the free receptors in the typhoid bacilli prepared according to the method of Neisser and Shiga, reported that very marked inflammatory local reactions followed the injection of 0.5 cubic centimeter of the prophylactic in the inoculation of some of the German troops. Redness and swelling of the tissues occurred three hours after the injection. The inflammatory area about the point of inoculation became of a scarlet-red color, sharply circumscribed, resembling the inflammation frequently seen in erysipelas. These manifestations subsided after forty-eight hours, but the injections were not repeated because of the marked local reaction which occurred from the first inoculation.

I wish to emphasize that these results obtained with extracts of the typhoid bacilli are entirely contrary to those which we have encountered with the free receptors of the cholera organisms. In very numerous inoculations performed in Americans and natives of these Islands we have had abundant opportunity to observe the local reactions following the inoculation of the prophylactic I have described; none of these have been severe and the great majority have been very mild. Our injections have been made intramuscularly because of the quick absorption which occurs from the muscular tissues. Since the immunizing substances in the extracted prophylactic are either in solution or colloidal suspension, they apparently are in a condition in which they are capable of being much more easily absorbed than when they are injected bound to the bacterial cells. However this may be, with the cholera organism the local reactions are unquestionably less severe after inoculation with our prophylactic than from the injection of the living spirillum. I have received inoculations by both methods, and the suffering caused by the living organisms is much more marked.

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[25] *Klin. Jahrbuch.* (1905), 14, 148, 156.

SIZE OF THE DOSE AND STANDARDIZATION OF THE PROPHYLACTIC.

Most observers, in discussing the size of the dose in inoculations against cholera, agree that the immunity obtained is in proportion to the amount of the immunizing substances injected. This was shown very conclusively by my early experiments which have been referred to in this article. However, Friedberger and Moreschi combat this view and, as has been mentioned, believe that as high an immunity can be obtained from the intravenous injection of very small amounts of the cholera spirillum as from the subcutaneous injection of much larger ones. I have not had any experience with the intravenous inoculation of the cholera organism in such small quantities as Friedberger and Moreschi have employed. The experiments of Fischera and of Meinicke, Jaffé and Flemming do not confirm Friedberger and Moreschi's results, as has been mentioned in the discussion of the literature; moreover, they believe that *with such small quantities of the organism* the very favorable results obtained may have depended more upon the individual variation of the animal in regard to susceptibility and immunity than upon the amount injected. In relation to the size of the dose inoculated it may be recalled that Wright,[26] in his inoculations against typhoid fever in the English army, attempted to inject each individual with almost exactly the same number of killed typhoid bacilli. In order to accomplish this purpose he employed a twenty-four. hour broth culture of a known and proved strain of *Bacillus typhosus* and enumerated the number of bacteria in this culture by the ingenious blood counting method which he devised.

Leishman and Harrison,[27] in further pursuing the question of typhoid inoculation among the English troops, also attempted to standardize their vaccine by the procedure advanced by Wright. However, they found that in spite of all precautions, errors in the broth cultures of from 50 to 100 per cent in counts of the same films were by no means uncommon. Leishman and Harrison also spent some time in their efforts accurately to standardize their typhoid prophylactic (consisting of the killed typhoid organisms,) by estimating in addition with the assistance of Martin, the weight of the dried bacterial bodies in a measured quantity of vaccine and a correlation obtained from this weight and the number of bacteria as estimated by the living and dead counting methods. Even by all these methods it was impossible finally to arrive at an accurate standardization such as they apparently desired.

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[26] *Brit. Med. Journ.* (1900), **1,** 122, and *Lancet* (1902), **2,** 11.
[27] *J. of Hyg.* (1905), **5,** 380.

In my opinion these experiments performed in the standardization of either typhoid or cholera prophylactic are superfluous and unnecessary. The most practicable and accurate method I have been able to devise for the standardization of a prophylactic of this nature is the determination of the degree of immunization within certain limits which will usually be produced by approximately equal quantities of it in an animal. Evidently, exactly the same amount of immunity in a series of inoculated animals is practically never produced even though the dose injected is exactly the same, owing to the natural individual variations in the immunity of the animals.

Having determined upon an arbitrary unit of immunization and upon the minimum number of units of immunity a given volume of the vaccine must usually produce in animals, so that the inoculation of the same amount will give rise to the production of a satisfactory quantity of immune bodies in a few human beings, it is only necessary always to employ for man the amount of vaccine which will give rise in the animal to at least the minimum determined number of units of immunity. Since individual variations in immunity in human beings are so marked, it probably does not make any practical difference whether one individual is inoculated subcutaneously with a few hundred or perhaps even a thousand more killed organisms than is another one. Thus, while for adults the regular dose of the extracted prophylactic we employ is 2 cubic centimeters (which must give rise to at least 10,000 units of immunity in a rabbit), nevertheless, the results obtained in human beings inoculated with the same lot of prophylactic and with the same dose were surprisingly variable. Moreover, they conclusively demonstrated that nothing of practical importance was to be gained in attempting to inoculate each individual of a large number of people in a community with exactly the same quantity of receptors. I have observed two individuals, each inoculated with the same dose of the same fluid prophylactic, one of whom developed forty times as great a quantity of antibodies as the other. Such variations in the immunity produced in human beings after inoculation are not uncommon.[28] The same conditions are sometimes encountered in the immunization of monkeys, but they are less variable in the other lower animals.

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[28] Agglutinins do not invariably develop in the blood sera of human beings inoculated either with our prophylactic or with the living cholera organism. Two physicians were each inoculated with 2 cubic centimeters of the same lot of our prophylactic. After ten days the blood serum of one showed an agglutinative reaction in a dilution of 1: 700, while that of the other gave practically no agglutinative reaction. At about the same time I was inoculated with 3 oesen of the living cholera organism, and although a very marked local and general reaction was obtained, my blood serum ten days later showed practically no agglutinative reaction against the cholera spirillum.

Lamb and Forster[29] have proposed to adopt Wright's method of standardizing typhoid vaccine by determining the amount of the vaccine which will completely neutralize or remove the amboceptor content of a fixed quantity of normal goat's serum. However, since the binding power *in vitro* of the receptors in the vaccine, for the amboceptors of the serum may perhaps not exactly represent the immunizing power of the vaccine in the animal body, we have preferred to employ the method I have described. This method of standardization of the prophylactic by the units of immunity it gives rise to is obviously not an accurate one, but it is sufficiently accurate for all practical purposes. In standardizing our smallpox vaccine we regard the reaction obtained in a monkey following inoculation with it as the most important test of its efficacy; the exact degree of the reaction (which varies with the natural variation in the immunity of the animals) is not so important so long as a distinct reaction is obtained. In standardizing our cholera prophylactic we also seek to obtain a certain reaction in the serum of the rabbit, following its inoculation. The exact degree of the reaction obtained, provided a certain limit has been reached, is obviously of less importance, for the reason already given.

IMMUNIZING POWER AND VIRULENCE OF THE ORGANISM.

Another point about which some further discussion seems necessary is that of the immunizing power of the cholera organism to be chosen for the preparation of the prophylactic.

Pfeiffer, Friedberger[30] and I[31] found that, with cholera spirilla, a greater immunity was obtained with the more virulent organism. Pfeiffer and Friedberger employed four strains in their investigations. My experiments were carried on with two strains of cholera spirilla of widely different virulence and I was able conclusively to show that the virulent organism, upon inoculation, produced a higher immunity and at the same time bound a greater number of amboceptors in a cholera immune serum than did the avirulent one. At the time of the publication of these experiments I stated that "these conclusions apply to the two strains of cholera spirilla employed in the foregoing experiments. Whether they will also hold good with other strains of this spirillum or for micro-organisms in general must be decided by further experimental work." The experiments of Meinicke, Jaffé and Flemming seem conclusively to show that with different strains of the cholera spirillum

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[29] *Scient. Mem. Med. and San. Off., India,* Calcutta (1906), **21**, 7.

[30] *Berl. Klin Wchnsch.* (1902), **39**, 581.

[31] *Publications of the Bureau of Government Laboratories, Biological Laboratory,* Manila (1904), **21**, 1, and *J. Exp. Med.* (1905), **7**, 229.

in vitro, the binding power of the organism for amboceptors in a cholera immune serum is independent of the virulence of the organism. In some instances they found that a virulent cholera culture was able to bind more amboceptors than an avirulent one, but in many instances the reverse was the case.

Their experiments referring to the relation between virulence and immunizing power are not numerous. This seems to me to be unfortunate, for by means of their method of examination and the large number of cultures which they studied, they were in a position to solve this problem conclusively. In fact, in the small number of experiments they record, only one instance is given in their table of results in which they found that 1/10 oese of a killed avirulent organism, when injected intravenously into a single rabbit, furnished a serum with a bactericidal value of 1: 1,000, while 1/10 oese of a killed, highly virulent strain in another animal produced a bactericidal value of only 1: 200. However, while the results of those experiments suggest that immunizing power is independent of virulence, nevertheless, in the inoculation with such small amounts of the organism, the individual variation in the immunity of an animal plays such an important rôle that it would not be prudent to draw any general conclusions from this single result. For example in this same series of experiments three other rabbits were inoculated with 1/10 oese of the cholera strain (number 74); one of these gave a serum of a bactericidal value of 1: 2,000, one of 1: 1,000, and one of 1: 400. Therefore, it would seem that further experiments are necessary before we can reach a final conclusion on this subject. This question in particular is not settled in regard to the inoculation of the living organisms of different virulence, and of the relative immunity produced. I[32] recently performed some experiments with living plague bacilli of different virulence and found that the more virulent organism furnished the greater immunity. However, as I worked with but three strains of this bacillus my experiments also can not be considered as entirely conclusive for other strains of the plague organism. It seems possible that the coefficient of growth of the spirillum may play some part in the degree of cholera immunity produced; that is, the virulent organism may multiply more rapidly after inoculation than the less virulent one, as Gotsclich and Wiegand found in cultures; this need not necessarily imply that a greater volume of growth is obtained with the virulent organism, in fact the individual spirilla may be smaller in size than in the case of the avirulent culture. The larger forms of the spirillum, it has been observed, are much more common in cultures of the avirulent strains than they are in those of virulent ones. It must be recalled that Haffkine in connection with the question of virulence and immunizing power emphasizes the fact that the more virulent cholera organism produces the greater immunity, and MacFadyan[33] states that cholera cultures of high virulence yield the most toxic and cultures of low virulence the least toxic juices, while in those instances in which the virulence had been allowed to diminish to such an extent that 2 platinum loops of a culture did not kill a guinea pig, the toxicity of the juices suffered a corresponding drop, 0.5 and even 1 cubic centimeter failing to kill, whereas the animal succumbed to acute infection from 0.1 cubic centimeter from a virulent culture. This led MacFadyan to conclude that virulence and toxicity were intimately related as regards the cholera endotoxin, inasmuch as increased virulence implied increased toxicity and vice versa.

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[32] *This Journal, Sec. B., Med. Sci.* (1907), **2**, 187.

Therefore, in preparing our cholera prophylactic we select an organism which is known to possess high immunizing value and in addition one of maximum virulence.

THE SERUM TREATMENT OF CHOLERA.

Recently the question of the serum treatment of cholera has again attracted attention owing to the studies of Roux, Brau, and Denier, Kraus and MacFadyan, and before entering into a discussion of the subject I will briefly review their results.

Brau and Denier[34] found that they were able to obtain a very active toxin from the cholera vibrio by growing this organism in a special culture medium consisting of *bouillon Martin gélatiné*, 45 cubic centimeters, normal serum of the horse 45 cubic centimeters, defibrinated blood 10 cubic centimeters, heated to 60° C. for three hours. By growth of the organism upon this medium they were able to obtain the toxin regularly and in increased amount. After four days' development the cultures had become liquified; hæmolysis occurred after twenty-four hours; after seven days they were filtered through paper and then through a Chamberlain F. candle. Certain precautions are necessary in order to obtain the toxin in satisfactory amounts. They advise that the serum be heated at 60° C. for three hours in order to destroy the substances antagonistic to the development of the cholera vibrio. The thermostat must be kept at a constant temperature, variations even of 1° interfering with the production of the toxin; the optimum temperature was found to be between 38° and 39° C. It is also necessary for the cultures to be well aërated and shaken each day. Finally, the strain of cholera spirillum employed must not have been passed through animals, since such a passage diminished the toxic power of the organism with great rapidity.

Following this method they were able to obtain a cholera toxin with 26 cultures of vibrios isolated in Saigon, with two strains obtained from the Pasteur Institute, one of which was isolated in Bombay and the other in Nasik, and with three strains from Egypt. They concluded that a soluble toxin may be obtained from vibrios isolated from cholera stools and that the production of the toxin may be increased by cultivating the organisms in their special culture media. In the following year[35] the same authors called attention to the fact that this cholera toxin manifested its effect quickly and without a period of incubation when injected into an animal. Guinea pigs and rabbits could be immunized against the toxin so that they were able to resist two fatal doses injected at one time, and horses which had been inoculated intravenously at intervals of 6 months with ½ liter of the toxin, furnished a serum of which 0.02 cubic centimeter neutralized two fatal doses of the cholera toxin after a contact of thirty minutes *in vitro*. The serum also exerted antimicrobic, agglutinating and precipitating qualities. The cholera toxin was not destroyed by boiling and the boiled toxin produced as good a serum as the unboiled one. It was also found that the injection of cultures of the living cholera vibrio into the veins of a horse furnished an antitoxic serum which was even more active than that prepared with the soluble toxin. They admit that the cholera toxin appears to be analogous to the endotoxins of the pest and typhoid bacilli, although in their final conclusions they state that the organism produces a soluble toxin the action of which is rapid and without a period of incubation. They also believe that the cholera toxin contained in the exudates of the bacteria and that obtained in the liquid culture media, can not be distinguished. The authors in their last article emphasize some further precautions to be observed in order to secure a good production of the toxin. The media finally employed consisted of 20 cubic centimeters of normal serum of the horse plus 10 cubic centimeters of defibrinated blood. The serum and defibrinated blood must be at least three weeks old before use, as otherwise almost no production of toxin occurs.

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[33] *Lancet* (1906), **171**, 495.

[34] *Compt. rend. Acad. d. Sc.*, Par. (1905), **141**, 397.

In 1903–4, in studying the question of protective inoculation against cholera, I called attention to the fact that judging from my experiments "it would appear that the most advantageous method for the extraction of the intracellular toxins of the cholera spirillum would be the one which MacFadyan has recently applied to the typhoid bacillus with the same end in view. By this method the bacteria were ground up at the temperature of liquid air, the disintegration having occurred under conditions which precluded the possibility of chemical change."

MacFadyan[36] during the present year, 1906, has undertaken experiments of this nature with sterile juices obtained from the cholera organism. Toxic extracts were obtained from the most virulent cultures which killed guinea pigs acutely in doses of 0.1 to 0.05 cubic centimeter while 0.02 cubic centimeter rendered the animals ill. The endotoxin also exerted its action when injected subcutaneously in quantities of 1 and 2 cubic centimeters. Doses of 0.1 to 0.05 cubic centimeter killed rabbits on intravenous injection. The juices deteriorated in toxic power on keeping, and the latter was destroyed by heating at a temperature from 55° to 60° C. Goats were immunized with increasing doses of the endotoxin and a serum was obtained of which 0.002 cubic centimeter neutralized from three to four ascertained lethal doses of the endotoxin for a guinea pig. This property was not possessed by 1 cubic centimeter of normal serum.

Kraus,[37] in 1904, in working with a vibrio designated as "Nasik," was able to obtain a powerful toxin from filtered bouillon cultures of this organism. By heating to 50° C. its poisonous properties were destroyed. Kraus concluded that his organism was not a true cholera vibrio owing to its agglutinative, bactericidal, precipitating, and haemolytic properties. Since this time the same author[38] has carried on extensive experiments with a number of different vibrios, which can not here be considered in detail. In his most recent article on the subject[39] he concludes that the cholera vibrio of Koch produces no haemotoxin either in buillon cultures or in goat's blood-agar plates. However, it gives rise to a toxin which is either produced by the spirillum only in the living organism, or also *in vitro*. The cholera poison is a true, soluble toxin and may be destroyed by antitoxin. It is to be differentiated from Pfeiffer's endogenous poison, which in the organism produces no antitoxin. Cholera is therefore an intoxication which is excited by a secreted, soluble toxin.

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[35] *Compt. rend. Acad. d. Sc.* Par. (1906), **142**, 728, and *Ann. d. l'inst. Pasteur* (1906), **20**, 578.

[36] *Lancet* (1906), **2**, 494.

[37] *Centrbl. f. Bakteriol. Orig.* (1904), **34**, 488.

[38] *Ibid,* (1906), **41**, 15, and *Wien. klin. Wchnsch.* (1906), **19**, 655.

A study of Kraus' experiments does not seem to me entirely to justify his conclusions. Moreover, his results are not altogether in accord with those of Brau and Denier. Kraus distinguishes two kinds of soluble poisons in the different spirilla, one, which is the most potent, acutely acting toxin, such as that produced by the *Vibrio nasik,* and a large number of other not true cholera vibrios, and a second which is a slowly acting, poisonous substance encountered in filtrates of true cholera cultures and which he regards as the toxin which gives rise to the cholera symptoms observed in man. Brau and Denier state that the toxin they obtained from the cholera vibrio acts acutely and without an incubation period, and that they secured this toxin from the *Vibrio nasik,* as well as from many other undoubted cholera strains. The toxin with which they worked was not destroyed by boiling, while the one which Kraus obtained from the *Vibrio nasik* was destroyed at a temperature of 50° C. However, Brau and Denier in their last publication on this subject incline to the belief that they formerly encountered two toxins, one of which was destroyed by boiling and the other not. Kraus has apparently lost sight of the fact that MacFadyan has obtained an anti-endotoxic serum of such potency that 0.002 cubic centimeter protected a guinea pig against three lethal doses of the cholera endotoxin, while Brau and Denier found that 0.002 cubic centimeter of horse's cholera immune serum, the animal having received 0.5 liter of toxin intravenously, was able to neutralize but two fatal doses of the toxin after standing one-half hour *in vitro*. This serum did not follow the law of multiples, as 0.05 of a cubic centimeter was necessary to neutralize 3 lethal doses of toxin, while 1 cubic centimeter was required to neutralize 4 doses.

I demonstrated in 1903 that 0.2 cubic centimeter of a cholera anti-endotoxic serum would neutralize 4 lethal doses of toxin, when mixed immediately before inoculation. I also found, as MacFadyan has since done, that a temperature of 60° C. destroys most of this primary poison, or at least converts the toxin into toxoid. It would appear that the toxin which Kraus has obtained and which he designates as a secretion of the organism and as a soluble toxin, is none other than the one with which Brau and Denier, MacFadyan, and myself worked and that it should rather be regarded as an endotoxin, for convincing evidence to the contrary at least has not yet been brought forward.

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[39] *Centrbl. f. Bakteriol.* Referate, (1906), **38**, Beil. 84.

During the year, opportunity was afforded to study the antitoxic serum of Denier and to witness its practical application in the treatment of Asiatic cholera. A request having been made by Dr. Denier to carry on the experimental serum treatment of cholera in the Government cholera hospital in Manila, I was called upon to examine the sera and report upon them before this method of treatment was undertaken. The two sera which Dr. Denier employed were of a different nature. One serum designated as "A" was prepared by injecting the horse with the cholera toxin entirely free from the bacteria, and the second one, "B," was produced by injecting the horse with the living organisms. These sera were, upon examination, found to possess specific agglutinative and bactericidal properties, serum "B" showing a much higher value in this respect. No study was made of the neutralizing power of the sera for lethal amounts of the filtered cholera toxin. Guinea pigs inoculated with 1 cubic centimeter of serum "B" and at the same time with 1 or even 2 oesen of a cholera vibrio, of which the lethal dose was 1/10 oese, survived the inoculation; however, when they were inoculated with 5 oesen and 2 cubic centimeters of the serum, they invariably succumbed. Pfeiffer's phenomenon seemed to have been complete, as was shown by the post-mortem examination of a number of these animals, since microscopic preparations from the exudate in the abdominal cavity showed no motile vibrios and the animals had apparently died rather from an intoxication than from an infection. However, these experiments obviously do not demonstrate whether death had occurred from the effect of the endotoxin contained in such a large amount of the spirillum (5 oesen) or from the effects of another soluble toxin.

Serum "B" was found to protect against larger doses of the living organism than serum "A" as was proved by testing the bactericidal power of the two sera. The bactericidal value of the sera was apparently, at all events so far as the living organisms were concerned, the most important factor in protecting the animals, at least up to a certain dose. In many of the animals which died and which had not received excessively large doses of the cholera spirillum, Pfeiffer's phenomenon was also found to be complete or almost so.

In all, 52 human cases of cholera were treated by Dr. Denier with the sera. In each instance a bacteriologic diagnosis of cholera was made by Dr. Denier and also by this laboratory, as was customary with all cases in the Government hospital. The injections of the sera were given intravenously and in large quantities, as much as 250 cubic centimeters in a liter of Hayem's solution being inoculated at a single dose. Following this primary inoculation, 100 cubic centimeters of serum were injected in an equal amount of saline solution every three hours until a reaction on the part of the patient occurred. The average amount of serum given was from 300 to 500 cubic centimeters, but in one case 1,000 cubic centimeters were inoculated in twenty-four hours. The cases in the hospital were treated alternately with serum, that is, every other case admitted received this treatment. Dr. Denier remained at the hospital day and night and was indefatigable in his efforts to treat and care for the sick. The injections of the serum were usually given very shortly after the time of the admission of the case to the hospital. Obviously, the patients were frequently in collapse at the time of their arrival. Dr. Denier[40] has prepared the following table which at a glance shows the results of the serum treatment.

	Number of cases.	Cholera spirillum not isolated from the stools.	Dead.	Recovered.	Percentage of mortality.
Controls	21	3	13	5	72
Serum "A" antitoxic	16	1	11	4	75
Serum "B" antimicrobic	5	----------	2	3	40

From this table it is evident, as Denier points out, that the cases which received the antitoxic serum were not benefited by it, the mortality being even higher than in the ones which received no serum. The number of cases which received the antimicrobic serum is too small to justify decided conclusions, although the mortality is much lower. Denier calls attention to the fact that liquid and frequent serous movements occurred shortly after the inoculations, with the patients who received intravenously a large amount of the serum in Hayem's fluid, these movements in volume approximately equaled that of the liquid injected. Therefore, he thought that possibly the antitoxic serum was not retained in the body for a sufficient length of time to accomplish its action and that it was perhaps excreted into the intestine and passed in the stool. He suggests that the injection of the serum might therefore, under proper aseptic conditions, be made with better results into the abdominal cavity. It has occurred to me that, if the inoculated serum was excreted into the intestine, more favorable results might perhaps be obtained, at least in the early cases, with a serum of higher bacteriolytic power, since, in the event of the excretion of the serum by the mucosa of the intestine, it would be brought into direct contact with the cholera spirillum. Probably such a serum would exert no favorable influences by its bacteriolytic properties in the later stages of the disease. It would be interesting to ascertain whether better results could be noted in the treatment of cholera by the use of the antitoxic serum prepared according to the method recommended by MacFadyan, since he has, according to published reports, prepared the serum of the highest endotoxic power.[41]

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[40] Report à Monsieur le Gouveneur Général de l'Indo-Chine, Hanoi, Saigon, Oct. (1906.)

As yet we have not by our own experiments, been convinced of the production in cultures of a *soluble* toxin by the cholera spirillum, even when it is freshly isolated from the cholera stool. On the other hand, it does not appear to me definitely to have been determined that the toxin which MacFadyan and others have obtained from the protoplasm of the organism, really is the toxin in the same condition in which it gives rise to all of the symptoms of the disease in man. At all events, it would appear that the cell juices which MacFadyan has isolated probably also, in addition to the pure specific cholera toxin, contain certain other poisonous substances. A more extensive routine study of the cholera vibrios isolated freshly from the cholera stools, as well as of the strains of *Bacillus dysenteriæ* encountered in dysentery fæces, in relation to the formation by them of soluble toxins, will be pursued in this laboratory as opportunity offers.

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[41] In a personal communication from Dr. Denier I am informed that Dr. Besredka has recently succeeded in preparing an antitoxic cholera serum which possesses a much greater value than did the serum with which Dr. Denier performed his experiments in the treatment of human cases of this disease during the preceding year.

OBSERVATIONS UPON TREPONEMA PERTENUIS CASTELLANI OF YAWS AND THE EXPERIMENTAL PRODUCTION OF THE DISEASE IN MONKEYS.

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PART I.

HISTORICAL.

Schaudinn and Hoffman(3) in May, 1905, announced their discovery of a spiral-shaped parasite in the lesions of syphilis, which they named *Spirochæte pallida.*

As *spirochæte,* Cohn, 1872, is an amended spelling of *Spirochæta,* Ehrenberg, 1834, the name *Spirochæte pallida* became *Spirochæta pallida.* In the same year Vuillemin(4) selected *Spirochæta pallida* as the type of a new genus which he called *Spironema,* the organism found in syphilis thus becoming *Spironema pallidum,* a classification accepted by Schaudinn in 1905. Further investigation developed the fact that the name *Spironema* had been previously employed to designate a genus of mollusks, and accordingly could not be used in this connection. Stiles and Pfender(5) proposed the name *Microspironema pallidum* for the organism but before their publication appeared Schaudinn(6) had proposed the generic term *Treponema* and the specific name *Treponema pallidum,* Schaudinn, which is the correct name of the parasite of syphilis.

In February, 1905, Castellani(7) while investigating the etiology of yaws at Colombo, Ceylon, discovered spirochætæ in the serum of yaws lesions, one of which resembled very closely *Treponema pallidum* in its morphology. In the announcement of this discovery, which appeared in the "*Journal of the Ceylon Branch of the British Medical Association,*" June 17, 1905, he named the organism *Spirochæta pertenuis,* but as it undoubtedly belongs to the genus *Treponema,* the correct name is *Treponema pertenuis* Castellani. Several papers by this investigator have since appeared (8, 9, 10, 11, 12) dealing with the etiology of yaws and a few confirmatory reports of the presence in the lesions of yaws of *Treponema pertenuis.*

Wellman(13), in South Angola, Africa, was the first to confirm Castellani's observations, finding the organism in scrapings from yaws lesions in one case.

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[1] P. M. Ashburn, captain and assistant surgeon, United States Army, and Charles F. Craig, first lieutenant and assistant surgeon, United States Army, constituting the United States Army Board for the Study of Tropical Diseases as they Occur in the Philippine Islands.

He was not aware of Castellani's discovery at the time. July, 1905, so that his observations amount to an independent discovery of *Treponema pertenuis*, although the organism was first seen and described by Castellani. In a supplementary note regarding the *spirochætæ* found in yaws, Wellman says (14): "It is significant that this observation which has been spoken of as one of the most important discoveries of recent times, considering the fact that the *Spirochæta pallida* has been found by Schaudinn in syphilis, and considering the relation said to exist between yaws and syphilis (15) should have been made almost simultaneously in two such widely separated countries as Ceylon and West Africa."

Further confirmations of the presence of *Treponema pertenuis* Castellani in the lesions of yaws have been published by Powell (16), Borne (17) and MacLennan (18). Powell and McLennan found the organism in but one case, but Borne encountered the treponema in nine of eleven cases examined, and the latter wrote Castellani (19) that he had found them in forty-nine out of fifty-nine cases. Connor (20) was unable to demonstrate *Treponema pertenuis* in two cases of yaws occurring in Manipur State, India, using Leishman's stain, the method advocated by Castellani.

Description.—The following résumé is compiled from the published descriptions of the organism by Castellani. The treponema were found by him in the serum from the nonulcerated lesions and in smaller numbers in the ulcerated lesions of yaws.

The majority of the organisms are extremely delicate, though some individuals are thicker and stain more intensely, but all are thinner than "*refringens*" or other spirochætæ with the exception of *Treponema pallidum* Schaudinn. The length varies from a few μ to 18 or 20 or more. Both extremities are often pointed, but forms are met with presenting blunt extremities or one extremity blunt and the other pointed. Rarely, one extremity may show a pear-shaped expansion or a loop-like formation. The organisms are spiriliform, the number of waves in the spiral varying, but generally being numerous, uniform, and of small dimensions; sometimes an organism is observed having uniform, narrow waves for a portion of its length, the remainder being almost or quite straight. Two organisms may be attached together end to end, or twisted about one another. Castellani has seen forms indicating longitudinal division, two organisms lying parallel, close together and united at one end; he has also observed a few chromatoid points scattered irregularly in some organisms. With Leishman's or Giemsa's stain the organism takes a pale, reddish tint. Castellani also found, in very rare instances, a few oval or roundish bodies 5 to 6 μ in length, and 4 to 6 μ broad, staining purplish or bluish with Leishman's stain, and containing chromatin, collected at one point or scattered throughout the bodies. He suggests that these bodies may be a developmental stage of the *Treponema pertenuis.* In the open ulcerative sores of frambœsia Castellani found, along with *Treponema pertenuis*, three varieties of spirochætæ, as follows:

1. A thick, easily stained form, identical morphologically with *S. refringens* Schaudinn.
2. A thin, delicate form, with waves varying in size and number, and blunt at both ends. He named this organism *S. obtusa.*
3. A thin, delicate form, tapering at both ends, which he named *S. acuminata.*

Castellani found *Treponema pertenuis* present in the lesions of yaws in eleven of fourteen cases. Regarding its morphological resemblance and its relation to *Treponema pallidum* he said in 1906 (21):

"The spirochætæ found in the non-open lesions and some of those found in open sores of yaws are, in my opinion, morphologically identical with the *S. pallida* of Schaudinn. This is also the opinion of Schaudinn himself who very kindly has examined several of my specimens, but that if future investigation will prove that yaws is a spirochætæ disease, the yaws spirochæte will have to be considered to be biologically different from the spirochæte of syphilis."

Careful and frequent inquiries among the medical officers of the Army and civilian practitioners in Manila, during a period of almost a year, had but confirmed the impression gained from the literature (1 and 2), that yaws is a rare disease in the Philippine Islands, when we were shown some cases at Parañaque, through the courtesy of Dr. Luis Guerrero. At the time of our first visit there we saw four cases and at subsequent visits four others, while we were informed by the patients and their friends that the disease is very common throughout all the region about Parañaque, and we have since heard of it as common in certain towns of Tarlac Province, Luzon, and in the neighborhood of Parang-Parang, Mindanao, and it is quite probable that it is frequently seen and well known by the natives in most parts of the Archipelago.

We have also seen five cases in San Lazaro Hospital, Manila, all in lepers. We have not had an opportunity to treat any case that we have seen, but we have examined all and in ten we looked for *Treponema pertenuis,* finding it in all of them.

Our examinations of yaws cases, which have been made at relatively infrequent intervals for the reason that we had none under our immediate control and supervision, embraced inquiries into the clinical manifestations of the disease. In this regard they brought out nothing new that is important and the description which we might give of the clinical appearance of the disease would not differ greatly from those of most recent authors and even from those of a century ago by Winterbottom and Bateman (22), except that we think the large, ulcerative lesions are probably due to secondary infections and should not be credited to pure yaws, any more than suppuration in syphilitic lesions should be attributed to *Treponema pallidum.*

The observations which we shall discuss herein consist principally, then, of studies of the fresh and stained serous exudate from yaws lesions, which contained the *Treponema pertenuis*, as described by Castellani and others. As the serum presented nothing peculiar or characteristic of yaws except the treponema, the great bulk of our work consisted in observations on that parasite. These observations were made on three varieties of preparations of the serum.

METHODS OF EXAMINATION.

A. Stained smears.—These were prepared by removing the yellowish, beeswax-like tops from the papillomatous lesions, either by pulling them off entire or by washing them off by friction with wet gauze, and taking on the end of a slide a bit of the clear serum which then exudes from the lesion and making a very thin smear of it across a thoroughly cleaned slide. Preparations so made were then stained with either Wright's or Giemsa's stain, preferably the latter.

A more profuse flow of serum is obtained if the crust or cap of the swelling be washed away, as the friction necessary in this process probably causes an increased flow of blood to the lesion; at any rate, a remarkably profuse flow is so obtained. On the other hand, if the cap be merely pulled off, the serous flow may be very slight and it only becomes profuse when the papilloma is rubbed with the end of a slide or with a piece of gauze, and even then it is not so abundant and so free from cells as the serum obtained by the first method.

It is remarkable how clean and how free from body cells and bacteria the slides thus made may be.

B. Fresh serum.—This was obtained in the same way as that for staining, except that it was generally allowed to flow into capillary tubes, whence it was blown out upon slides and diluted or not, as seemed desirable, with a small amount of normal saline solution. It was then covered with a thin cover glass, the glass ringed with vaseline and examination made with a high power.

C.—Capillary tube preparations were made as indicated above, the tubes being sealed when filled and kept at room temperature (usually about 30° centigrade) for variable lengths of time, when they were broken, the contents blown out and examined stained or unstained, or both.

In addition to the examinations made of the serum, in the ways just indicated, we excised two papillomata and sectioned and stained them by Levaditi's method. We were unable to demonstrate the *Treponema* in these preparations, but in sections similarly stained and sent to us by Captain Russell, acting curator of the Army Medical Museum, they are seen in great numbers, lying among the epithelial cells, but less numerous among the deeper layers of these. The organisms are in many instances aggregated into clumps similar to those obtained in the capillary tubes. The cells among which the organisms are found always show signs of degeneration, loss of outline, indistinctness of nuclei, and vacuolation. Such areas are localized and present the appearance of lacunæ of degeneration.

DESCRIPTION OF TREPONEMA PERTENUIS CASTELLANI.

a. Morphology.—The morphology of the treponema may be very briefly described by the statement that it is indistinguishable, so far as we can determine, from that of *Treponema pallidum.* In shape, size, staining reactions, appearance of ends, etc., the two are similar, and neither we nor the many medical men and investigators to whom we have shown the organisms and whose opinion we have sought, are able to differentiate them.

In length *Treponema pertenuis* varies considerably, some short forms not being longer than about 4 μ. It is possible that other forms may be even shorter than this, but if so they are not recognizable as treponema. More commonly they are about 10 to 12 μ in length, while individuals are even longer. Occasionally very long forms are seen, 20 and 25 or very rarely even 30 μ, but whether these are single individuals, or multiplying or agglutinating forms in which two individuals are joined end to end, we can not yet determine.

The width of the organism is so very slight that we are unable to measure it with exactness. We estimate its width as probably varying from one-sixth to one-half of a micron. If the line of a filar micrometer is brought as near to one side of a loop of the treponema as is possible without covering the latter, and the line of the instrument be moved $\frac{1}{8}$ or $\frac{1}{4}$ μ toward the treponema, the side of the loop will be hidden in most instances. Whether it will be completely covered, or more than covered by the line, we can not say. We can not be more definite than to say that in our opinion the width of the organism is not far from 0.25 μ, but it may be either greater or less. The length of the spiral turns averages very close to 1.5 μ, measured from crest to crest. When we first began to study the organism we thought that *Treponema pertenuis* was probably a trifle wider and a trifle more open in its curves than *Treponema pallidum*, and we yet think that this may possibly be true for the average of large numbers, but our average measurements are the same for both, and there is no form that we have seen which we felt justified in designating as either *pallidum* or *pertenuis*, one and not the other, unless we knew the source from which it was derived.

The curves of both species of treponema vary somewhat in width and regularity, but these variations are not peculiar to or even much more common for either species. In general, the curves are fine, about 1 to 1.2 μ in depth, regular and rather rigid. The last-named character is especially noticeable in unstained, fluid preparations. Here the organisms are seen as fine and fairly rigid spirals, usually straight or almost so. The appearance is the miniature of that produced by a long spiral wire spring. Such a spring may be bent by slight pressures, but it at once resumes its straight form when the pressure is relieved, and in either the straight or the bent form it retains its spiral turns.

This description applies particularly to fluid preparations from a few hours to a few days old. In quite fresh preparations the treponema can not usually be seen, or if seen, recognized. Flashes of very motile organisms may be observed, and it is a fair presumption that some or all of them may be treponema, but the motion is so rapid and the glimpse of the organism so fleeting that no deductions can be drawn as to morphological characteristics.

It is important to note that in fluid preparations the morphology of the organisms is much more regular than in stained ones, and it is therefore probable that many of the variations in the latter class may be due to the drying and staining process. This statement applies to our experience with both *pallidum* and *pertenuis* and we think that it lessens the value of the deductions based solely on the morphology of stained specimens.

However, since the described morphology of both *Treponema pallidum* and *Treponema pertenuis* heretofore rested almost entirely on the descriptions of stained specimens, it is well to consider such specimens here; but it should be borne in mind, that no matter how many shapes, sizes and forms the stained organisms may show, there is not one of them which can not be imitated by the use of the spiral wire spring to which we likened those in the wet preparations, if the spring be subjected to forces analogous to those acting on the treponema during the making and drying of the smear.

The stained forms, presenting many variations as to size and shape, may be most conveniently described by dividing them into types or classes. While the length and number of curves vary greatly, the examination of a large number of *Treponema pertenuis* shows the average number of curves to be about eight. Seventeen is the greatest number counted on one organism, two the smallest. Many individuals show only four or five turns, only a few have more than twelve. All types, shapes and sizes stain with difficulty, showing best with Giemsa's stain, which gives them a pinkish-violet color.

Type A (see Pl. IV) is probably the most common stained type of *Treponema pertenuis*, as it is of *Treponema pallidum*. This may be said to be the classical type of the latter, but the other forms to be described for *pertenuis* may also be found for it.

This type is usually straight, or but slightly bent; it shows regular and even curves which are very fine, and terminates in narrow pointed ends which have been interpreted as flagella. It stains evenly throughout, although the finely pointed ends show less distinctly than the main part, probably because of their size.

Type B is likewise very common, and differs from A in having a straight and usually thin portion in some part of its course, frequently near the middle. This appearance has been variously interpreted by different observers; principally as a union of two organisms by their flagella in beginning agglutination, as an incomplete separation of individuals resulting from longitudinal division, or as beginning or incomplete transverse division.

A spiral wire spring may be made to present a similar appearance if one or more of its coils is straightened out by traction and pressure.

Type C corresponds most closely with that we consider the unaltered form as seen in wet preparations. It corresponds to type A except that instead of terminating in finely pointed ends, it shows a dot or little knob at one end or often one at each end. This is the appearance seen almost uniformly in wet preparations and we think that it is due to a tight curling of the ends, such as is seen on the shoots of many young plants. This curl may straighten out under the influence of age or drying, and the end may then appear as a finely attenuated point or flagellum.

Type D is also common and differs from A and C in that one or both ends, instead of showing a finely attenuated point or blunt dot, show a ring.

This ring frequently appears thicker and heavier than the main part of the body. With the spiral wire we may obtain a similar picture if we turn up an end or the ends of the spiral so as to look down through part of it and view another part from the side. That such bends should frequently be caused by the spreading and drying of the serum is readily to be believed for the reason that we often see the same thing entirely in profile, that is, the spiral turns are still preserved, but the whole spiral so bent as to form two or three sides of a quadrangle.

Type E may present the terminal features of any of the above but it also shows in its continuity a complete, circular loop, or more than one. The spiral wire assumes a similar appearance if one turn be pressed down or back.

Type F represents a short, loosely curved organism attached to a ring or two rings, one at either end. This, we think, differs from D only in the proportions of the spiral viewed from the end, and in the fact that the part viewed in profile has the regularity of its curves more altered by pressure or drying.

Type G represents various irregular forms which differ from the more characteristic individuals in form only, not in size or staining power. It is possible to produce all of them by pressure or traction applied in various ways to the wire spiral.

Type H embraces the individuals which show dots in some part of their continuity. These have been referred to by various writers as chromatin dots, as representing nucleus, blepharoplast, etc. Whether or not they be such, or are merely kinks or twists in the organism, we do not know. We are unable to determine any constancy in the frequency, number, or localization of their occurrence, and an analogous appearance may be caused in the wire coil by kinking or twisting it.

These various types may be found pure or in a great variety of combinations, such as B C, G D, C D H, H G, A H, etc.

Types A, B and C are seen in the wet preparations, and of the three, C is by far the most common. It is unusual to see in such preparations any individuals which do not show the knob-like ends and the regular curves throughout their length. The other forms D, E, F, G, and H we have rarely or never seen in wet preparations, but occasionally we have observed individuals, particularly in old ones, which presented a somewhat beaded appearance that might represent type H of the stained specimens.

MOTILITY.

In neither fresh nor stained specimens have we seen anything that we interpreted as an undulating membrane, nor anything that was differentiated as a flagellum in the distinct manner in which flagella are differentiated on certain bacteria or *trypanosomata*. The motility of the organisms as seen in wet preparations probably varies from the extremely active movement already mentioned, which permits one to see a flash of glancing light but no more, to a very sluggish motion which so closely simulates entire passivity as to leave the observer in serious doubt as to whether any is present, other than that due to currents in the serum.

When motion has ceased to a sufficient degree to permit of the organism being well seen and clearly identified, it is always slow. It consists of a slight rotation on the axis of the spiral representing the appearance of a corkscrew movement, and a mild and gentle waving and bending of the entire organism. These two varieties of motion combined, cause the treponema to pass across the field, to rise and sink, necessitating much change of focus, or, if one end of the organism it attached to the cover glass, or to foreign matter in the field, as is frequently the case, to lash or to swing in an indolent manner from this fixed point.

This motion and what we consider the common form of the organism may best be observed in capillary tube preparations about one day old. In that length of time the accompanying bacteria have usually not multiplied so greatly as to occupy the large part of the field they do later, while the treponema have ceased to move actively, and have usually increased in number and are readily found. In these preparations, or stained ones made from them, what are most commonly called the dividing forms are more frequently seen.

DIVIDING FORMS.

For ease of description we may designate these forms as additional types I, K, L and M.

Type I is fairly common and, as indicated by Plate IV shows some variety in the arrangement of its component parts. Essentially it consists of two or more spirals which are attached one to another by their ends. In some, this attachment is such as to be almost or quite indistinguishable from type B.

Type K is also quite common and differs from I in that the attachment is firmer and involves a greater part of the length of the parasite. It is as though the wire of the spiral had been split throughout a quarter, a half, or three quarters of its length, the turns being preserved. However, the two sections of the spiral are frequently unequal in length. The appearance presented in such cases has been figured by Siedlecki and Krystalowicz(23) as representing conjugation.

Type L is probably the most striking and pretty form to be seen. Here the two component parts are intertwined throughout their length, the two ends at one extremity, however, being free. Occasionally all four ends are free, but more commonly those at one extremity are fused, or they take their origin from a common dot or knob.

These types, I, K, and L, have usually been considered indicative of longitudinal division, and they so appear to us.

However, some writers, particularly Novy and Knapp(24), have considered it possible that division of *Treponema* may be by transverse fission, and type M, which is very rare, would seem to be an example. In this type we see what looks like one organism but it shows a break in its continuity. More than one break rarely occurs. Whether these apparent breaks are really such, or are merely artefacts, we do not know. They usually appear like the latter. We have seen the appearance indicated both in stained preparations and in photomicrographs, but not in wet specimens.

VIABILITY.

We have found treponema which showed slight motion and preserved their forms in a capillary tube preparation of serum made thirty-four days prior to the time of its examination; apparently the organisms were alive but we made no inoculations with them. The bacteria, which had been very numerous in other preparations made at the time, but not kept so long, had so far as we could see all died, leaving a pure culture of *Treponema pertenuis*.

Undoubted motion is preserved by organisms in tube preparations for a period of several days, although it is always sluggish after a few hours and often after one hour or less.

CULTIVATION.

At the suggestion of Dr. Miyajima we studied capillary tube preparations of the serum from yaws lesions, in the hope of obtaining agglutination of the treponema, as Dr. Miyajima said that he had obtained it in similar preparations from chancres. Our hopes in that direction were speedly realized, as we obtained marked clumping in the first one we made. These tubes were examined on the second, third and fourth days.

We have not since had an opportunity to discuss the matter with Dr. Miyajima, and therefore are not quite sure as to what he meant by the term "agglutination." If he used it as meaning merely the aggregation of already existing organisms into clumps, we think that his statement was not sufficiently broad, as it is our opinion that the numbers of treponema in such preparations are greatly increased after one day to a week.

Usually, the increase is manifest within 24 hours; however, at times, it takes place more slowly and may only become well marked after several days. The increase in numbers and the clumping are both almost, but not quite, constant; of the two increase is, in our opinion, the more so. We obtained a similar result in one of the two cases of syphilis in which we made the same experiment with serum from the chancre.

It is difficult to determine accurately whether a smear preparation containing scattered treponema has as many organisms as another preparation in which they are in clumps. Novy and Knapp, who describe agglutination of *Spirillum obermeieri* say that in the case of that organism there is no increase in number. Counts being out of the question, the observers' estimate must be relied upon. Our belief, based on many examinations of sera made on the first, second, and third days, etc., is that in most instances *Treponema pertenuis* multiplies greatly when kept in the serum in capillary tubes, and in some instances the increase seems to occur without the agglutination. Our opinion that the organisms multiply in such preparations is based not only on the greater number of them found in the tube preparations as compared with smears made at the same time, but also on the appearance of the clumps and the great preponderance of what we consider dividing forms as described under types I, K and L. The nature of some such clumps and dividing forms are shown in the accompanying photomicrographs. (Pl. 1, figs. 5 and 6.)

Exceptionally, neither multiplication nor agglutination developed in the tubes, and the failures, while infrequent, are somewhat irregular and do not admit of what we consider a thoroughly satisfactory explanation. However, in general terms, we are of the opinion that they are due to one or both of the following reasons:

First. Variability in the immunity of the yaws patient and in the amount of antibodies contained in the serum; possibly the variability of immunity may even be local, or toxins elaborated by bacteria may be present locally to inhibit the growth. An interesting observation which may bear on this point was made in a case of syphilis.

Serum from a chancre was taken up in tubes on Monday. The chancre was then washed with bichloride solution and dressed with calomel. On Tuesday it was thoroughly cleaned and more serum placed in tubes. The tubes of Monday showed marked increase and clumping of treponema, those of Tuesday, neither. The patient was not taking any general treatment.

Second. Variability in the bacterial content of the tubes and consequently in their content of soluble toxins.

While it is readily possible to make fresh smear preparations of yaws serum which show very few or no bacteria, it has not been possible, in our experience, to obtain a serum really free from these organisms, and in the sealed tubes they multiply enormously. However, the fact that both multiplication and clumping occur in the great majority of tubes encouraged us in the idea that we might cultivate the treponema indefinitely, and as the favorable medium seemed to be the serum of susceptible persons or animals, we endeavored to obtain growth on monkey blood and on ascitic fluid from a patient who was suffering from cirrhosis of the liver who also gave a history of syphilis. The ascitic fluid was used in two ways, plain and heated to 60° C. for thirty minutes to destroy complement. In none of these media did we obtain any growth of the treponema, although bacteria developed in all.

However, we did not have the opportunity to repeat these experiments, and we are not convinced that cultivation of the treponema is impossible.

PATHOGENESIS.

It has been stated by different authors that yaws is inoculable on lower animals, notably cats and monkeys, and that it is inoculable from person to person. We have made no inoculation experiments on persons, and none on lower animals other than monkeys (*Cynomolgus philippinensis* Geoff.). Of these we inoculated five, using serum from the yaws of three different patients. All five of the monkeys developed yaws lesions of a sufficiently characteristic appearance to permit of diagnosis based on that feature alone.

In addition to this typical appearance we found *Treponema pertenuis* in all of the lesions. The organisms did not differ in any demonstrable way from those seen in serum from human lesions. In numbers, measurements, staining reactions, shape and motion they were similar.

However, the monkeys did not show the secondary lesions of a generalized infection, nor could we, in the instance in which we tried it, induce yaws in other monkeys by inoculating them with the blood or splenic juice of an infected animal. The yaws lesion did spread, and in that way give rise to what might be termed secondary lesions, but this was always by continuity and we observed no evidence to make us think that it was ever through a general blood or lymph infection.

BIOLOGICAL POSITION OF TREPONEMA PERTENUIS.

We see no reason to doubt that the biological position of *Treponema pertenuis* is as close to that of *Treponema pallidum* as one species may be to another. The almost overwhelming weight of scientific opinion at the present time seems to leave the latter organism where Schaudinn placed it, among the Protozoa.

However, its protozoal nature is not universally accepted, and probably will not be for some time to come. Our opinion is that both organisms are protozoal, but while so eminent a zoölogist as Stiles(25) concedes to others the right to regard *pallidum* as of vegetable nature, we feel that we may safely grant the same latitude in respect to *Treponema pertenuis*. What we believe to be more immediately important and more easily determinable are the following propositions:

First. That *Treponema pertenuis* is constantly found in the serum from yaws lesions.

Second. That it can, at the present time, be differentiated from *Treponema pallidum* only by the consideration of the lesion from which it is obtained, or by the inoculation of certain animals.

Third. That its many forms in stained preparations are all explainable on the supposition that it is a regular spiral, often deformed by the forces or processes concerned in the spreading, drying and staining of the smears.

Fourth. That, as will be shown more fully in Part II of this paper, the inoculation of serum containing this organism causes yaws in monkeys, and that the organism is again found in the lesions of the inoculated animals.

Fifth. That *Treponema pertenuis* is the cause of yaws.

PART II.

THE EXPERIMENTAL PRODUCTION OF YAWS IN MONKEYS.

Historical.—The literature relating to the production of frambœsia in monkeys by the inoculation of material from the lesions of the disease is very limited and so far as we have been able to determine Neisser, Baerman and Halberstädter, working together in Batavia, Java, and Castellani, in Colombo, Ceylon, have been the only investigators to produce the disease in these animals. To Castellani belongs the credit of demonstrating *Treponema pertenuis* in the experimental lesions in monkeys, the other investigators mentioned not searching for the organism, although, in their report they mention Castellani's discovery of a spirochæta in the lesions in man.

Neisser, Baerman and Halberstädter (26) inoculated seven monkeys with serum from yaws papules, the inoculation being made upon the breast and over the eyebrow, by rubbing the infective material into small abrasions in the regions mentioned. Frambœsia developed in all of the animals, the incubation period varying from thirteen to fourteen days in two *Gibbons*, to 96 days in *Macaccus*. In the latter, five in all, the incubation period was twenty-two, thirty-one, sixty-five, ninety-one and ninety-six days, respectively. So-called secondary lesions developed in three of the animals, forty, forty-nine and seventy days after the primary lesions had appeared, the authors stating that the secondary lesions always appeared upon the site of the initial one and extended in a serpiginous manner into the surrounding skin. They did not observe a generalized eruption in any of the animals. They also inoculated seven monkeys (*Macacus nemestrin, M. niger* and *M. cynomolgus*) with material from yaws papules in monkeys suffering from the disease. In only one of these animals (*M. niger*) inoculated from a *M. nemestrin,* did the disease develop after an incubation period of thirty-four days.

The authors then endeavored to produce the disease in monkeys by subcutaneous inoculation of a mixture of splenic juice, bone marrow and lymph glands from a *Gibbon* suffering from yaws. They injected three *M. cynomolgus,* with negative results in all. Inoculation of three monkeys of the same species with splenic pulp and three with the bone marrow from an infected *M. cynomolgus,* resulted in one of the three inoculated with bone marrow developing frambœsia after an incubation period of forty-four days.

These investigators also demonstrated that monkeys successfully inoculated with syphilis developed frambœsia upon inoculation. In one instance a monkey (*M. niger*) was inoculated upon April 17 with syphilis and developed the primary syphilitic lesion upon May 13. On May 28 the same monkey was inoculated with frambœsia and the typical yaws papule developed at the site of inoculation upon August 1. Another monkey (*M. cynomolgus*) was inoculated September 23 with frambœsia, and on October 25 with syphilis. Upon November 8 a typical yaws papule appeared at the site of inoculation, while on November 15 the characteristic syphilitic lesion appeared.

From their experiments the authors mentioned draw the following conclusions:

1. Frambœsia is inoculable from man to higher and lower apes.

2. Frambœsia is inoculable from ape to ape.

3. Infection in monkeys following inoculation of the bone marrow proves that frambœsia is a general and not a local disease.

4. Apes infected with syphilis are susceptible to frambœsia.

Castellani(27) inoculated four Ceylon monkeys (species not given) with frambœsia. Only one of these developed the disease after an incubation period of nineteen days. A small papule, which enlarged slowly and became covered with a crust, developed at the site of inoculation. Two months later, the original papule being still present, four others appeared, two upon the forehead near the first lesion, and two upon the upper lip; one of these papules disappeared in a few days, but the other three enlarged slightly, became moist and a yellowish crust formed over each. At the end of three months all of the lesions had healed. In the scrapings from the lesions, *Treponema pertenuis* was demonstrated repeatedly.

Six weeks after the disappearance of the yaws lesions this monkey was inoculated with syphilis, and sixteen days later a typical syphilitic lesion developed, accompanied by general glandular enlargement.

The positive results obtained by the investigators whose work we have briefly reviewed, led us to repeat partially their experiments with a view of determining if the native monkey of the Philippines, *Cynomolgus philippinensis* Geoff., could be infected with frambœsia, and of adding, if possible, something to our knowledge concerning the disease as observed in these animals and the relation of *Treponema pertenuis* to the lesions produced by experimental inoculation. While our observations can not be considered as completed, we believe the results so far obtained are of interest and should be put upon record. In the main, our work has confirmed the results of the above mentioned investigators and we believe we are justified in asserting that frambœsia is easily inoculable from man to monkeys and that *Treponema pertenuis* is constantly present in the active experimental lesions and stands in a causal relationship to them.

Material and methods.—The monkeys used in our experiments were all *Cynomolgus philippinensis* Geoff., the common native monkey of the Philippine Islands. We have experimented with eleven monkeys, the inoculations in such animals being both by the subcutaneous pocket method and by vaccination, that is, rubbing a little of the yaws serum into slight abrasions upon the skin. The site of inoculation was generally the skin of the abdomen and forehead, but the inside of the thigh was used in inoculating with syphilitic serum. The method by vaccination proved, in our experience, slightly more successful than the subcutaneous pocket method, but it is probable that if a larger number of animals were used there would be found to be no difference in the results obtained. In every instance of successful inoculation, the slight wounds healed rapidly and the site of inoculation appeared normal until the development of the yaws papules at periods varying from sixteen to thirty-five or forty-five days after the inoculation. The serum used in making the inoculations was obtained in the manner already described for securing smears for staining.

In searching for *Treponema pertenuis* in the lesions in monkeys the same methods of securing specimens of the serum and staining them were used as have been already described in Part I of this report.

We also endeavored to secure cultures of *Treponema pertenuis* from the lesions in monkeys, using methods similar to those employed in our endeavors to cultivate the organism from human lesions.

In considering the experimental production of frambœsia in these animals we were desirous of investigating many problems intimately connected with the subject, aside from the mere successful result of inoculation, and while we have attempted to solve some of them, we do not feel justified as yet in expressing an opinion regarding our results in certain directions. This is especially true of our experiments regarding re-infection and the local or general nature of the disease as it is observed in monkeys in general, for our experiments in these directions are too few to be of definite value, although they are suggestive. The following protocols of our inoculations include those already completed and those in which it is too early as yet to predict the result.

PROTOCOLS OF THE EXPERIMENTS.

Monkey No. 1 (3070).—This monkey was inoculated on February 16, 1907, with serum from a typical yaws papule on a young Filipina girl. A subcutaneous pocket inoculation was made in the skin of the abdomen, and some of the serum was rubbed into a scarified area over the left eyebrow. Smears of the serum, prepared at the time the inoculation was made, showed numerous examples of *Treponema pertenuis.* The inoculation wound healed rapidly and the animal appeared normal until March 4, when a small papule covered with a yellowish crust was noticed at the point of the inoculation upon the abdomen; the crust was removed and smears made from the serum which exuded from the minute granulations. An examination of these smears demonstrated the presence of *Treponema pertenuis* in large numbers. The period of incubation was about sixteen days. Upon March 8, a small, crusted papule had appeared at the point of inoculation over the left eyebrow and smears of serum from this lesion also showed *Treponema pertenuis.* Both the lesions enlarged slowly, especially the abdominal one, and healing in the center, extended in a circular manner into the surrounding healthy skin. The lesion upon the head had disappeared by May 14, but the abdominal lesion persisted until May 28. Duration of the disease, eighty-two days. On May 15, this animal, still showing a yaws papilloma upon the abdomen, was inoculated with serum from a chancre which contained numerous *Treponema pallidum.* A subcutaneous pocket inoculation was made in the skin of the abdomen and in addition some of the serum from the chancre was rubbed into an abrasion upon the inside of the left thigh. No results have followed these inoculations to date, June 30, 1907.

Monkey No. 2 (3071).—On February 16, 1907, this animal was inoculated subcutaneously on the abdomen and through an abrasion over the left eyebrow with serum from a yaws tubercle from the same case as monkey No. 1 (3070). On March 8, a small, crusted papule was observed at the site of inoculation on the head, which gradually enlarged until it reached the size of a small hazelnut; a typical, yellowish crust developed, which upon removal disclosed the characteristic, pink, granulating surface of a yaws papilloma. Examination of the serum from the lesion demonstrated repeatedly the presence of *Treponema pertenuis.* The incubation period in this case was twenty days. The lesion had entirely disappeared on May 2, thus making the duration of the disease fifty-six days. On May 16 this animal was reinoculated with yaws serum through a subcutaneous pocket upon the abdomen and an abrasion over the right eyebrow. No lesion has appeared to date, June 30, upon the abdomen, but the inoculation wound over the right eyebrow suppurated and a deeply excavated ulcer resulted. Repeated examinations of the material from the ulcer have always resulted negatively for *Treponema pertenuis.*

Monkey No. 3 (3072).—This animal was inoculated February 26, 1907, with serum from a yaws tubercle on a native woman, the inoculation being made upon the abdomen and over the left eyebrow in the manner described. The serum used contained many examples of *Treponema pertenuis.* On March 8 after an incubation period of twenty days, a small, crusted papule was noticed at the site of inoculation upon both the abdomen and the head. Both lesions enlarged, became covered with a typical crust, and the examinations, which were made repeatedly, were always positive for *Treponema pertenuis.* This monkey did not stand close confinement well, became weaker and weaker, and was chloroformed on March 18, ten days after the appearance of the yaws lesion. At autopsy the viscera appeared normal, but the cervical and inguinal glands were slightly enlarged. The yaws tubercle upon the abdomen measured 1 by 0.75 centimeters, was considerably raised above the surrounding skin and covered with a yellowish crust. The lesion upon the head was 1.5 by 1 centimeter, and was very typical of the yaws papilloma as seen in man. The pathologic material was handed to Dr. H. T. Marshall, pathologist of the Bureau of Science, for examination.

Monkey No. 4 (3073) was inoculated February 26, 1907, upon the abdomen and over the left eyebrow in the manner described, with serum from a yaws tubercle in a native woman. Upon March 8, twenty days after inoculation, a typical yaws papule developed at the site of vaccination upon the head. This lesion enlarged slightly, became covered with the characteristic crust, and the examination of the serum from the granulation tissue revealed upon the removal of the crust showed the presence, repeatedly, of a few *Treponema pertenuis.* By April 16, the lesion had healed, the duration of the disease being about thirty-nine days. On May 15, this animal was inoculated upon the abdomen and right thigh with serum from a chancre showing many examples of *Treponema pallidum.* No results have been obtained from this experiment to date, June 30, 1907.

Monkey No. 5 (A).—This animal was inoculated April 10, 1907, upon the abdomen and right eyebrow, with serum from a yaws tubercle in a leper woman, the inoculation upon the head being subcutaneous, upon the abdomen by rubbing the serum into a slight abrasion. No lesion appeared upon this monkey until May 29, when a well-developed papule about 1 centimeter in diameter and covered with a thick, yellowish crust was observed. This lesion had evidently existed for several days, so that the incubation period is uncertain, probably between thirty-five and forty-five days. Upon removal of the crust the typical pink, raspberry-like growth was well marked and examination of the serum from the lesion demonstrated *Treponema pertenuis.* In this case the lesion enlarged but slightly and by June 12 had disappeared, the duration of the disease being about two weeks.

Monkey No. 6 (3109).—This monkey was inoculated on March 18 by the subcutaneous pocket method upon the abdomen and through an abrasion upon the forehead, with blood from the heart of monkey No. 3 (3072), obtained at the time of autopsy. No lesions have developed in this animal to date, June 30, 1907.

Monkey No. 7 (3110).—Was inoculated March 18, 1907, in the same manner as monkey No. 6 (3109) with splenic juice from monkey No. 3 (3072) obtained at autopsy. No lesions have developed in this animal to date, June 30, 1907, but there is marked enlargement of the inguinal lymphatic glands.

Monkey No. 8 (3111).—Inoculated as above with serum from the yaws lesion upon head of monkey No. 3 (3072) obtained at the time of autopsy, March 18. This animal was in a weakened condition from continued confinement at the time of inoculation and died on April 6, nineteen days after the inoculation. No lesions of yaws had appeared at the time of death and the autopsy did not show anything of interest beyond enlargement of the spleen, liver, kidneys and the lymphatics of the abdomen.

Monkey No. 9 (B).—This animal was inoculated April 13 in the manner already described with yaws serum from a leper woman, the serum having been kept in a glass capillary tube for three days. No lesions have appeared in this monkey to date, June 30, 1907.

Monkey No. 10 (C).—Inoculated through a subcutaneous pocket upon the abdomen May 15 and through an abrasion upon the inside of left thigh, with serum from a chancre, showing very numerous examples of *Treponema pertenuis.* No lesions have appeared to date, June 30, 1907.

Monkey No. 11 (D).—Inoculated May 15 in the same manner as monkey No. 10 (C) with serum from a chancre showing the presence of *Treponema pallidum.* No lesions have appeared in this monkey to date, June 30, 1907.

The two latter animals were used as controls to our inoculation of syphilis in yaws monkeys No. 1 (3070) and No. 4 (3073).

SUMMARY.

The protocols given show that, in all, eleven monkeys have been used in our experimental work. Of these, five were inoculated directly with serum from human yaws lesions; one with serum from a human lesion, the serum having been kept in a glass capillary tube for three days; one with blood from the heart of a monkey that had developed yaws; one with splenic juice from the same monkey; one with serum from a yaws papilloma in a monkey; and two with serum from a primary syphilitic lesion. In addition, one monkey after recovery from yaws was reinoculated with human yaws serum, and two after recovery were inoculated with syphilis. As regards results; of the five monkeys inoculated with yaws serum taken immediately from the human lesions all developed typical yaws tubercles; the animal inoculated with serum from a yaws lesion in a monkey died before the period of incubation, as shown by our experiments, had expired; the monkey reinoculated with yaws after recovery has developed no lesions. Lastly, in not one of the four monkeys inoculated with syphilis have any lesions developed.

PERIOD OF INCUBATION.

As will be seen upon referring to the protocols, the period of incubation of yaws in the monkeys we experimented with varied from sixteen to about forty-five days, but it should be understood that this is only approximate, as owing to the distance of the location of the animals from us and pressure of work, the animals were not inspected every day and thus the lesions may have existed a short time before they were noticed. However, the limit of error in this respect is small and of no practical importance. The approximate period of incubation in our five successful inoculations was as follows:

	Days.
Monkey No. 1 (3070)	16
Monkey No. 2 (3071)	20
Monkey No. 3 (3072)	20
Monkey No. 4 (3073)	20
Monkey No. 5 (A)	35 to 45

In the case of monkey No. 5 (A), the yaws lesion, when first noticed, was about the size of a small pea and had obviously been present for a number of days.

Comparing our results with these of Neisser, Baermann and Halberstädter(28), it is noticeable that in our monkeys the period of incubation was much shorter, as a rule, although the same low type of animal was used. Indeed, the incubation period of yaws in *Cynomolgus philippinensis* Geoff., approaches more nearly that in Gibbons, as is shown by the investigators mentioned. Thus, in the lower types of monkeys used by them, the incubation period in five animals was found to be twenty-two, thirty-one, sixty-five, ninety-one and ninety-six days respectively, while in only one of our five animals did it probably exceed twenty days. If we add to this result the probability that the lesions in all of our cases may have existed for a day or two before they were noticed, thus shortening the period of incubation still further, the difference in our results and those in the German commission becomes more noticeable. The regularity of the period in our animals is also worthy of notice, four of them developing the disease between the fifteenth and twentieth day after inoculation.

Duration of the disease.—In the five monkeys in which we produced frambœsia by inoculation the duration of the lesion was as follows:

Monkey No. 1 (3070), eighty-four days; No. 2 (3071), fifty-seven days; No. 3 (3072), ten days (this animal was chloroformed while the lesions were still active); No. 4 (3073), thirty-nine days; No. 5 (A), fourteen to twenty-one days.

It was invariably our experience that in the more severe cases the primary lesion tended to spread into the surrounding skin, and the more marked this tendency was, the longer the disease lasted. We failed to observe any general glandular enlargement or any symptoms pointing to a general infection.

The lesions of frambœsia as observed in monkeys.—The lesions produced by the experimental inoculation or frambœsia in monkeys do not differ essentially, in their morphology, from those occuring in the disease in man, but we have never observed the secondary or generalized eruption, which, according to most authors, follows the primary lesion in the human subject. Neisser, Baermann and Halberstädter regard as secondary eruptions the extension of the infection from the site of the original lesion and in one of our animals, Monkey No. 1 (3070), such an extension occurred. However, we do not believe the new lesions so produced to constitute a secondary eruption, but simply to be an invasion of the contiguous healthy tissue by the organism causing the disease; that is, the treponema. Castellani appears to have secured true secondary lesions situated at a distance from the original papule in his one successful inoculation, and in this case a general infection might be supposed to exist.

The evidence obtained from our experiments would appear to indicate that experimental frambœsia in the monkey, at least in *Cynomolgus philippinensis* Geoff., is a purely local infection which readily heals after a period of time varying from a few days to several weeks. As we have stated, a few days after inoculation, the wounds had completely healed, although when the infection was conveyed by means of a subcutaneous pocket a slight thickening about the site of inoculation persisted for a short time, finally disappearing before the appearance of the yaws papule.

In all of our animals the yaws lesion appeared at the site of inoculation and when first diagnosed consisted of a small papule, very slightly elevated above the surrounding skin and covered with a yellowish cap or crust. The papules varied in size from that of a large pin's head to a small pea. The epidermis had been replaced by the yellowish crust, which upon removal revealed a moist surface composed of minute, closely aggregated, but separate, pinkish points, from which a thin, slightly milky fluid exuded.

The initial papule gradually enlarged, became in most instances elevated, and the crust, formed of the exuded serum, became thicker and more noticeable. The lesions were circular in form and firm upon pressure. Even when fully developed they were not greatly elevated, as is so frequently the case in human yaws tubercles, and in only one of our animals did they project markedly above the surrounding surface. While the crust covering them was always more or less elevated, it would almost invariably be found upon its removal that the granulating surface was but slightly raised, although very distinctly demarcated from the healthy skin surrounding it.

The crust covering the fully developed yaws lesion in the monkey was perfectly characteristic of that over similar lesions in man, varying in thickness, easily removed, and yellowish-brown in color, sometimes streaked with reddish-brown due to admixtures with blood.

The surface of the fully developed yaws papule in the monkey, after the removal of the crust, was typical of that observed in human lesions. The color varied from a light pink to a bright red, and a colorless or slightly whitish serum oozed from the raw surface which consisted of minute, closely aggregated papillæ, situated upon a slightly raised base and surrounded by apparently healthy skin. In some of our animals the typical "raspberry" appearance, so characteristic of the human yaws tubercle, was well illustrated. When fully developed the papules averaged 1 centimeter in diameter. In one of our animals, Monkey No. 1 (3070), the lesion both upon the head and the abdomen was typical of that variety of the disease described by Pierez(29), Scheube(30), Manson(31) and others as "ringworm yaws." The first lesion appeared upon the abdomen and presented the appearance already described. After a few days it was observed that in both the abdominal lesion and that which had meanwhile appeared on the head, healing was occurring at the center while the edges were covered with an elevated crust. At this time the lesion resembled a ringworm infection so closely that we made an examination for the fungus, with negative results.

The lesion upon the head, when fully developed, measured about 2 centimeters in diameter and consisted of a perfect ring of raised, granulating tissue covered with the characteristic yellowish crust, and inclosing the original site of the yaws papule, which had healed without scar formation and but little pigmentation. Removal of the crust disclosed the usual moist, pink surface and an examination of the serum exuding from it demonstrated the presence of *Treponema pertenuis* in large numbers. A slight extension of this lesion occurred in the form of a small, characteristic papule developing at its lower portion and slightly involving the eyelid.

The abdominal lesion enlarged rapidly and for some time presented the appearance of a large yaws tubercle, markedly elevated and covered with a mammillated yellow crust. Healing began at the center of the tubercle and soon a typical, "ringworm" appearance was assumed but here a very considerable invasion of the surrounding skin occurred, new papules appeared at the periphery of the original lesion, so that eventually nearly one-half of the surface of the abdomen was involved in the process. The new lesions were easily demonstrated to be extensions, in direct continuity with preëxisting ones and sound skin was never found separating these lesions while in the active stage. Their progress answered perfectly to the so-called secondary lesions described by Neisser, Baermann and Halberstädter, but as we have stated, we can not regard them as an evidence of a general infection and therefore as "secondary" in the sense in which the term is used in connection with syphilis.

After persisting for a varying period of time, the lesions of frambœsia heal in the same manner as those occurring in man, the hypertrophied papillæ atrophy, the crust covering the papilloma shrivels up and falls off, and a slightly discolored, but apparently sound area of skin, devoid of hair, marks their former site. After a few days the hair again grows and it becomes practically impossible to discover the point of the inoculation. As it is now nearly three months since our animals have recovered from the infection, and as we have seen no evidence of a generalized secondary eruption, we believe we are justified in asserting that in the species of monkey we used, a general eruption of yaws does not occur after experimental inoculation. While the lesions of frambœsia are undoubtedly modified somewhat in the monkeys of the low type used in our work, they are yet so characteristic that we believe, from their appearance alone, a clinical diagnosis could be made even in the mildest case of infection we have observed, while in the more severe infections, such as Monkey No. 1 (3070), the nature of the lesion was apparent at a glance. It is probable that if higher species of apes were used, the lesions would be much more pronounced and a generalized eruption of yaws tubercles might occur.

Examination for Treponema pertenuis.—We have examined the lesions in all of our sucessfully inoculated animals for *Treponema pertenuis* and have repeatedly demonstrated its presence in every case, without any special difficulty. The organisms occurred usually in the very earliest stage of growth of the yaws papule and persisted until the lesion had nearly healed, being most numerous during the active growth of the papule and decreasing in number as the healing process advanced. As we have stated, the treponema occurring in the lesions in monkeys did not differ in any particular from the ones found in the serum from the lesions in man. In most instances no other spirochætæ were observed in the preparation, although, in one or two cases, organisms corresponding to the type of *S. refrigens* were observed, but these were very rare. As in man, the lesions covered with crust showed the treponema unmixed with other spirochætæ, while in those in which the crust had been removed, for instance by scratching, thus allowing secondary infections to occur, organisms corresponding to the types described by Castellani were infrequently observed.

Serum from the lesions in some of our inoculated animals was collected in capillary tubes and kept for varying periods of time. Apparent multiplication of *Treponema pertenuis* occurred in some, and the organisms remained motile for several days. In the material so collected the organisms occurred singly, in pairs, or in clumps. Agglutination and apparent longitudinal division were also observed in the serum from the lesions in these animals.

We consider the constant presence of *Treponema pertenuis* in the experimental lesions of yaws in monkeys, produced by the inoculation from the lesions in man, of serum containing them and their absence in other conditions, to be conclusive proof of their etiological relationship to frambœsia. If we add to this the fact that as the lesions heal, the treponema gradually disappear and the further fact, as proved by the case of Monkey No. 2 (3071), that the organisms can not be found in pyogenic ulcerations even when inoculated, unless frambœsia be induced, it appears to us that the evidence is complete. *Treponema pertenuis* is found constantly and only in the lesions of frambœsia, whether they are natural, as in human infection, or experimental, as in the infection of animals.

Reinfection.—In only one instance [Monkey No. 2 (3071)] have we attempted to reinfect a monkey that had recovered from frambœsia, and in this animal the reinoculation of human yaws serum resulted negatively.

Inoculation from monkey to monkey.—In one instance [Monkey No. 8 (3111)] we attempted to inoculate a monkey with the serum from a well-marked lesion occurring in another animal of the same species, but unfortunately the inoculated animal died in nineteen days, before the probable period of incubation had expired. In view of the results of Neisser, Baermann and Halberstädter, who obtained only one successful result from the inoculation of seven monkeys with the serum of infected animals, it is obvious that no conclusions can be drawn from our single experiment.

Inoculation of blood and splenic pulp.—In order to determine whether frambœsia, as observed in infected animals, is a general or local disease we inoculated one monkey [No. 6 (3109)] with blood from the heart of an animal infected with yaws, and another [No. 7 (3110)] with splenic pulp from the same animal. No results followed these inoculations, but we do not consider that the experiments prove anything as Neisser, Baermann and Halberstädter obtained only negative results in six monkeys inoculated with splenic pulp and with a mixture of splenic juice, bone marrow and mesenteric glands and only one positive result in three animals inoculated with bone marrow. We did not attempt the inoculation of bone marrow, but in view of the fact, that of the nine animals injected by the investigators mentioned, the only positive result was obtained by the inoculation of this substance, we feel that our negative result with the blood and splenic pulp does not justify us in drawing a definite conclusion as to the production of the disease in this manner.

Inoculation of yaws and syphilis.—Both Castellani(32) and Neisser and his co-workers appear to have proved conclusively that monkeys which have recovered from yaws are susceptible to syphilis. We have endeavored to repeat their experiments, but have failed to produce syphilis either in monkeys which have recovered from yaws or in those that have never suffered from the disease.

As shown in the protocols of our experiments, we inoculated two animals, Monkey No. 1 (3070) and Monkey No. 4 (3073), both of which had recovered from well-marked yaws lessions, with serum from a chancre containing at the time of inoculation numerous examples of *Treponema pallidum.* As controls we inoculated two healthy animals with serum from the same case. At the present time, two months after inoculation, none of these monkeys has developed syphilitic lesions, and we are forced to the conclusion that it is extremely difficult, if not impossible, to inoculate syphilis in the species of monkeys used in our experiments (*Cynomolgus philippinensis* Geoff.). This difficulty, compared with the ease with which frambœsia in transmitted to the same species, speaks very strongly against the identity of the two diseases.

Yaws and syphilis.—As is well known, the question of the relation of yaws to syphilis has always excited much controversy, and Hutchinson's theory that yaws is the original form of syphilis, the latter disease, as we observe it to-day, being frambœsia modified by passage through the Caucasian race, still has many supporters. The discovery of an organism in yaws lesions indistinguishable morphologically from *Treponema pallidum,* at first sight appeared to lend additional evidence to the claim that yaws and syphilis are identical, but the experimental evidence already at hand demonstrates that the lesions produced by *Treponema pertenuis* differ greatly from those caused by *Treponema pallidum,* and that infection with one of these organisms does not produce immunity against the other. *Treponema pertenuis* and *Treponema pallidum* are, therefore, distinct species, and the lesions produced by each are characteristic and easily distinguished clinically, in uncomplicated cases.

There is no room for doubt in our minds, after consulting the work of other authors and investigators and our own clinical and experimental experience, that yaws and syphilis are distinct diseases, our belief being based upon the following facts:

(*a*) The pleomorphism of the lesions of syphilis, the uniformity of those of yaws.

(*b*) The granulomata (yaws papules) are the primary lesions of yaws; such lesions, if syphilitic, could only be *secondary* or *tertiary.*

(*c*) The presence of the very peculiar and typical yellow cap, or crust, covering the yaws lesions.

(*d*) In infected regions every uncomplicated case of yaws, whether in children or adults, presents the same characteristic lesion (the papule covered with a yellow crust.) If the disease were syphilitic a wider variation in the type of the lesion would be observed.

(*e*) The epidemic occurrence of yaws, especially among young children, and the greater prevalence of the disease in children.

(*f*) The absence of genital infections in any case observed by us.

(*g*) The absence in yaws of such striking symptoms as loss of hair and iritis.

(*h*) The auto-inoculability of yaws, even when a general eruption is present.

(*i*) The ready inoculability of yaws into such a low type of monkey as *Cynomolgus philippinensis* Geoff., and the negative result of the inoculation of syphilis in this species of monkey.

(*j*) The fact, as proved by Neisser, Baermann, and Halberstädter, and by Castellani, that monkeys susceptible to both yaws and syphilis can be infected with both, no immunity being conferred against the one by an attack of the other.[2]

(*k*) The fact, as proved by Charlouis (33) and Powell (34), that patients suffering from yaws can be infected with syphilis.

GENERAL CONCLUSIONS.

As a result of our observations, both clinical and experimental, we believe that we are justified in drawing the following conclusions:

1. That *Treponema pertenuis* is the cause of yaws.
2. That *Treponema pertenuis* is constantly present in the serum from yaws lesions.
3. That the variations in the morphology of *Treponema pertenuis* are explainable by the deformities produced during the preparation of the serum for examination.
4. That *Treponema pertenuis* and *Treponema pallidum* can be differentiated by the results obtained from the inoculation of monkeys.
5. That the inoculation of the serum from human yaws lesions containing *Treponema pertenuis* causes yaws in monkeys and that the organism can easily be demonstrated in the lesions of the infected animals.
6. That the length of the period of incubation in *Cynomolgus philippinensis* Geoff. is approximately twenty days.
7. That the duration of the inoculated disease in this species of monkey varies from twenty-one to eighty-four days.
8. That yaws and syphilis are distinct diseases.
9. That *Treponema pertenuis* can be demonstrated in sections of yaws papillomata by the Levaditi method.

Castellani, in an article published in the Journal of Hygiene for July, 1907, and only reaching here after the preceding paper had gone to the printer, draws the following summary and conclusions:

"*1.* Monkeys are susceptible to yaws. The skin eruption in the monkeys I have experimented with (*Semnopithecus priamus* and *Macacus pileatus*) is, as a rule, confined to the seat of inoculation, but the infection is general, as is proved by the presence of the *Spirochœta pertenuis* in the spleen and lymphatic glands.

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[2] On September 6, 1907, all of the experimental monkeys, except the two mentioned above as dead and monkey No. 2 (3071), which was killed, were examined and none of them showed signs of either syphilis or yaws. One of the monkeys utilized in the experiments detailed in this report [No. 2 (3071)] was killed on July 22, 1907, because of the extension of the pyogenic ulcer on his brow to the orbit. All of the others are still living on October 7, 1907, and one of them [No. 11 (D)] has given birth to young. No one of them has shown signs or symptoms of either yaws or syphilis since they were last noted in the report.

"*2.* Material obtained from persons suffering from yaws and apparently containing *Spirochœta pertenuis* only is infective to monkeys.

"*3.* When the *Spirochœta pertenuis* has been removed from this material by filtration, the latter becomes inert.

"*4.* The inoculation of blood from the general circulation and blood taken from the spleen of yaws patients into monkeys may give positive results.

"*5.* The inoculation of the cerebro-spinal fluid of yaws patients gives negative results.

"*6.* Monkeys successfully inoculated with yaws do not become immune for syphilis.

"*7.* Monkeys successfully inoculated with syphilis do not become immune for yaws.

"*8.* By means of the Bordet-Gengou reaction it is possible to detect specific yaws antibodies and antigen.

"*9.* Yaws antibodies and antigen are entirely different from syphilitic antibodies and antigen.

"*10.* The presence of the *Spirochœta pertenuis* in monkeys experimentally inoculated, as well as in yaws patients, is practically constant in the unbroken eruptive lesions; the *Spirochœta* is frequently present in the spleen and lymphatic glands.

"*11.* Yaws is generally conveyed by actual contact, but under certain circumstances it may be conveyed by flies and possibly by other insects."

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ILLUSTRATIONS.

PLATE I.

FIG. 1. *T. pertenuis* from human yaws lesion. 1 × 1500 (approximate).
2. *T. pertenuis* from human yaws lesion. 1 × 1500 (approximate).
3. *T. pertenuis* from human yaws lesion. 1 × 1500 (approximate).
4. *T. pertenuis* from human yaws lesion. 1 × 1200 (approximate).
5. *T. pertenuis* from capillary tube culture, showing agglutination and probable longitudinal division. 1 × 1200 (approximate). Note comparative size of treponema and cocci.
6. Same. 1 × 1500.

PLATE II.

FIG. 7. *T. pertenuis* from capillary tube culture, showing agglutination and probable longitudinal division. 1 × 1500 (approximate). Note comparative size of treponema and cocci.
8. *T. pertenuis* from inoculated yaws in monkey. 1 × 300 (approximate).
9. *T. pertenuis* from inoculated yaws in monkey. 1 × 1500 (approximate).
10. *T. pallidum* from human syphilis. 1 × 1500 (approximate).
11. *T. pertenuis* in degenerated area of epithelium of human yaws lesion. Levaditi method.

PLATE III.

FIGS. 12–15. Examples of yaws lesions as seen in Filipinos.

PLATE IV.

Diagrammatic illustration of types exhibited by *T. pertenuis* in stained preparations.

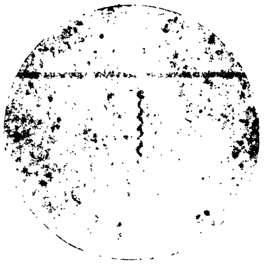

FIG. 1.

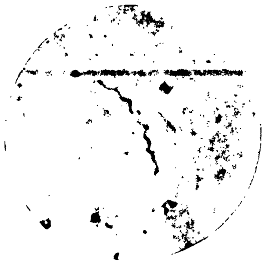

FIG. 2.

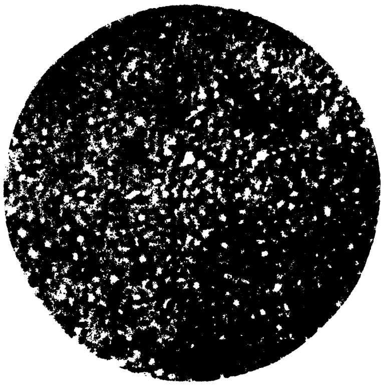

FIG. 3.

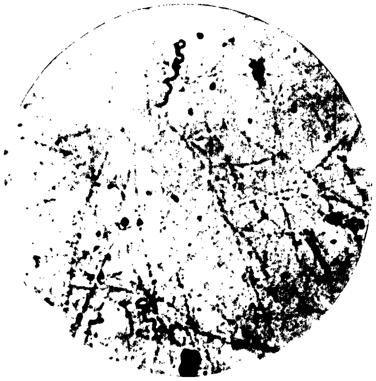

FIG. 4.

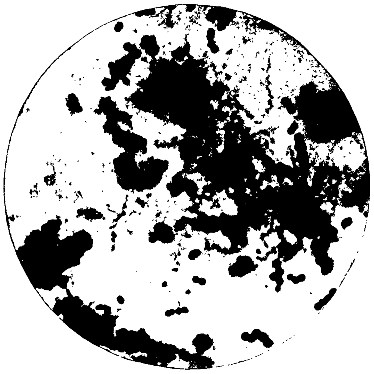

FIG. 5.

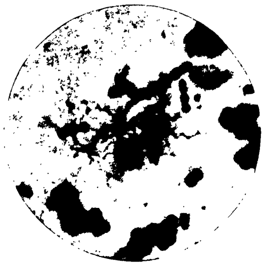

FIG. 6.

PLATE I.

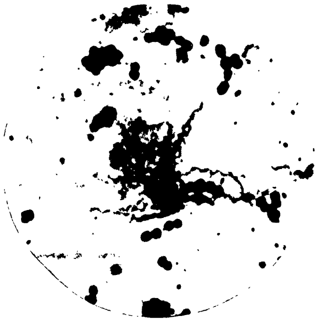

Fig. 7.

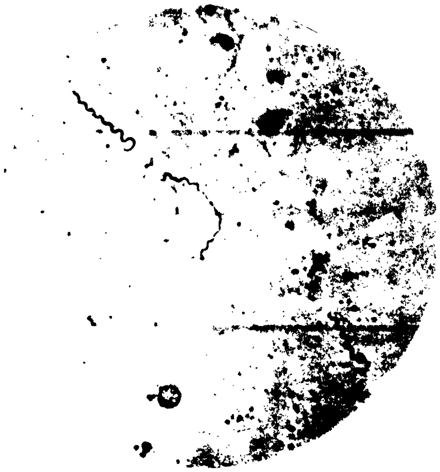

Fig. 8.

Fig. 9.

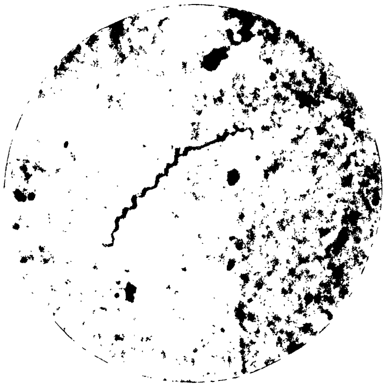

Fig. 10.

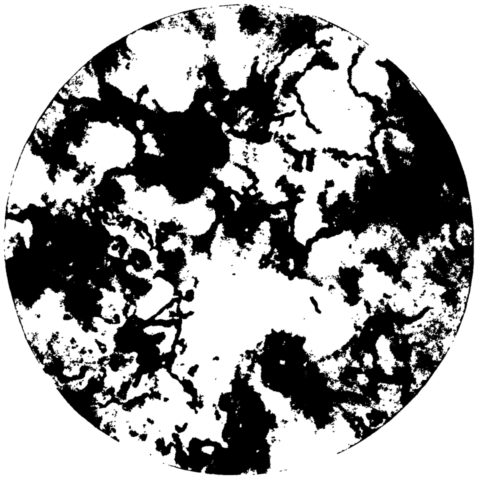

Fig. 11.

PLATE II.

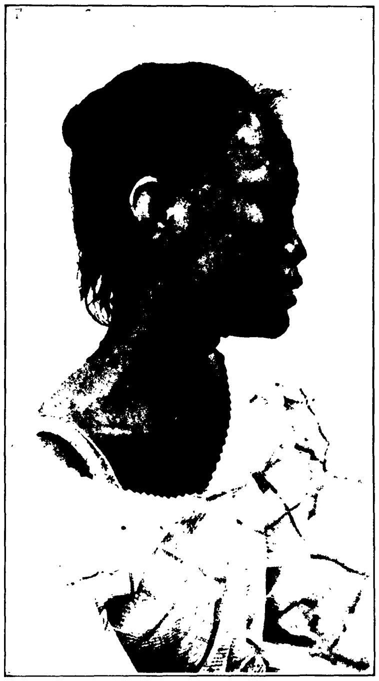

FIG. 12.

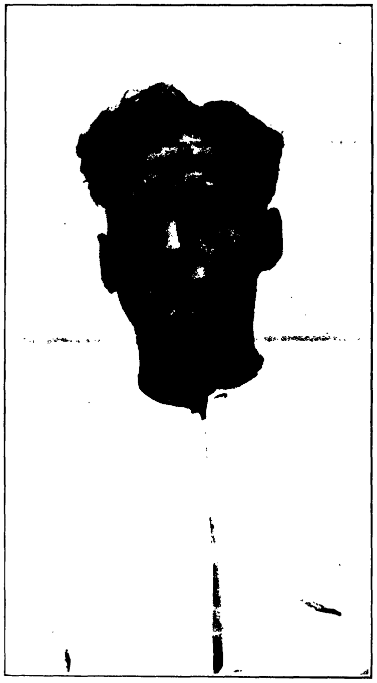

FIG. 13.

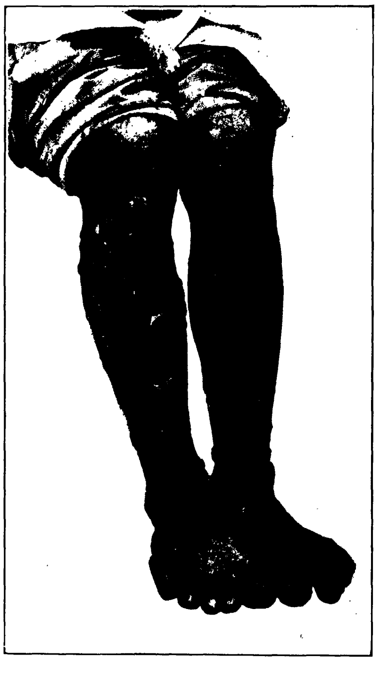

FIG. 14.

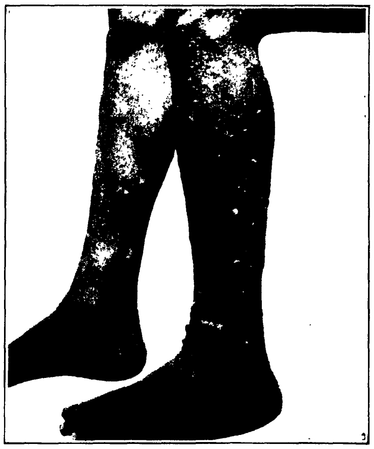

FIG. 15.

PLATE III.

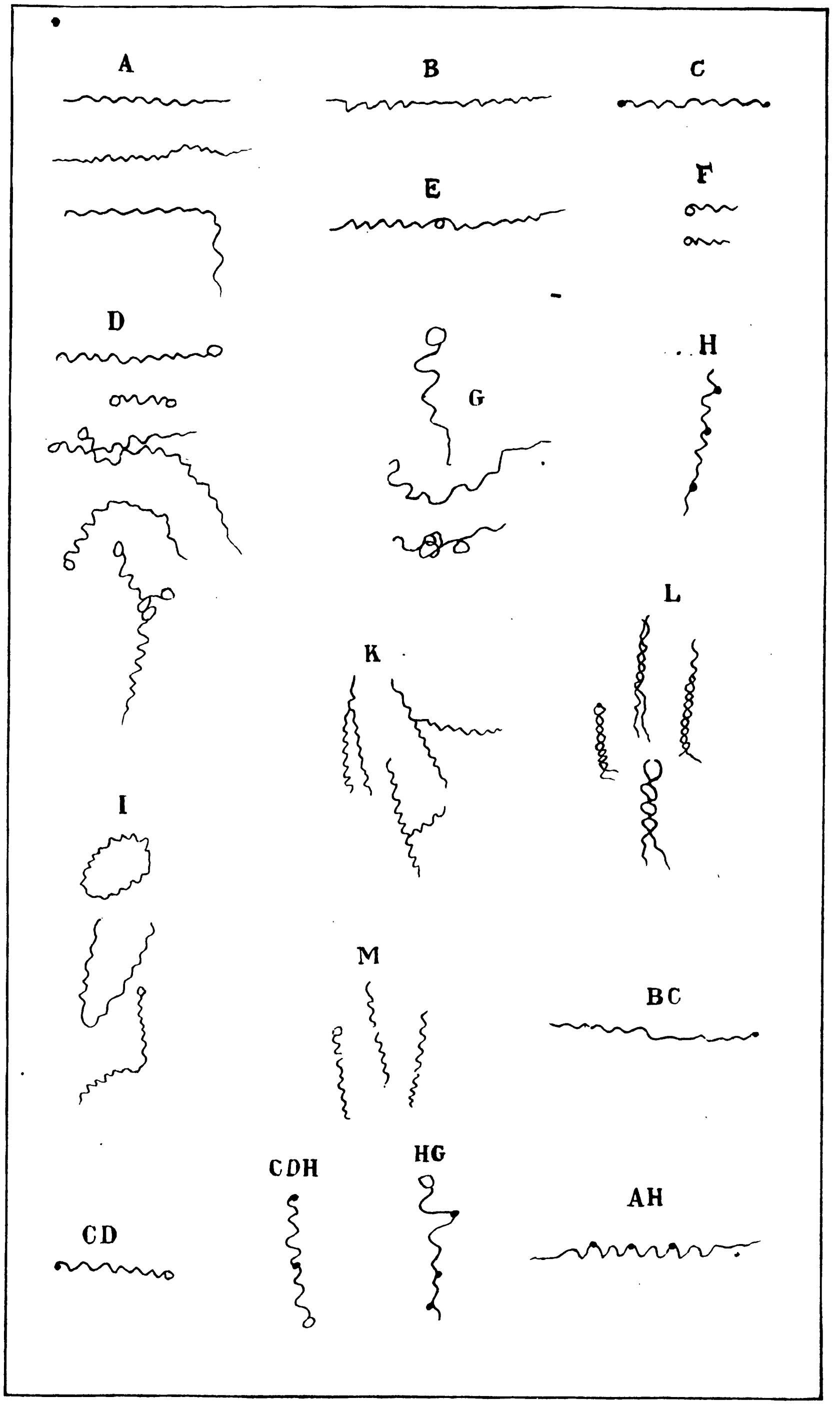

PLATE IV.

YAWS: A HISTOLOGIC STUDY.

By Harry T. Marshall.
(From the Biological Laboratory, Bureau of Science.)

Doctors Ashburn and Craig kindly turned over to me the tissues from the cases discussed in the preceding paper, for study. The tissues examined consisted (1) of an early yaws papule removed from a patient suffering with leprosy and yaws, and (2) of ulcerating yaws nodules from a monkey inoculated from a patient seen near Manila.

As the photographs illustrate, the lesions in the two cases are essentially alike and a single description suffices for them.

Sections through the papule and ulcers were fixed in Zenker's fluid immediately after removal and were treated with the ordinary stains. Hæmatoxylin and eosin gave the clearest pictures. The sections show (*a*) regressive, (*b*) vascular and exudative, and (*c*) regenerative changes. The regressive ones are confined to the epithelial structures; the exudative are most marked immediately beneath the epithelium; the regenerative changes are evidenced as atypical epithelial formations and by the presence of a few epithelioid connective tissue cells.

(*a*) The degenerative changes are confined to the epithelial structures, including surface epithelium, epithelial downgrowths, hair follicles, sebaceous and sweat glands. There is at first an increase in the thickness of the epithelium affecting chiefly the polyhedral cells, with blurring of the outlines between the separate layers and disappearance of the pigment layer. The horny layer is thrown off and the epithelium appears as a greatly thickened structure, made up of large, swollen, cloudy, vaculated cells, with swollen, pale nuclei, showing irregular dots of chromatin. The columnar cell layer is the only one that is well preserved. Where the disease is more advanced, the surface layers have been thrown off, exposing either the columnar layer of cells or the subjacent corium. The ulcer is coated in places with a crust of necrotic material, leucocytes, etc. (See Pl. I, fig. 1, *a, b, c, e.*) Similar changes appear in the epithelial downgrowths, the hair follicles, and to a lesser extent in the sweat glands; the centrally placed epithelial cells being swollen, very cloudy, vacuolated, and often replaced by cavities containing a few leucocytes and a small amount of granular débris, while the peripheral one or two rows of cells preserve fairly well their columnar shape, alignment, and staining properties. (See Pls. I and II, figs. 1 *c; 2 b;* 3.) Cross sections through an epithelial downgrowth or hair follicle with central softening, may be slightly suggestive of the "pearls" of epithelioma. (See Pls. I and III, figs. 1 *e; 2 c;* 4.) Charlouis, Unna, and others note especially that the hair follicles and hair shafts are unaffected, but this does not hold true of the nodules now under consideration. The sections from the human case did not pass through any hair follicles, but in the sections from monkeys, hair follicles are abundant and the outer root sheath shows exactly the same necrotic changes as are met with in the other epithelial structures. The sweat glands and sebaceous glands are altered less than the hair follicles and downgrowths.

(*b*) The vascular and exudative changes are marked in the corium, and extend only a short distance into the heavier strands of subcutaneous tissue. There is a very great dilatation of the capillaries extending to the under surface of epithelium. Many capillaries are empty, others contain polymorphonuclears, eosinophiles and a few mononuclears, while others are full of erythrocytes. At first sight it appears as if most of the erythrocytes are free in the tissue, but in my sections I have been able to make out a limiting capillary wall in most cases and find only small extravasations. The corium is markedly œdematous, the œdema extending into the epithelium and for a slight distance into the subcutaneous tissue. The early yaws papule contains many leucocytes and the ulcerating nodule is packed with them. A few polymorphonuclears are found in the epithelial structures, while the downgrowths and other degenerating parts are surrounded with collections of polymorphonuclears, large and small mononuclears, many of which are plasma cells, with occasional eosinophiles. A few lymphocytes are found and only a small number of extravasated erythrocytes. The eosinophiles have polymorphous nuclei. The leucocytes are also found both within capillaries and surrounding them, and it is most probable that they have arrived by way of the blood vessels and have wandered out from them. The distribution of eosinophiles is interesting. At the edge of the nodule, beyond the line of epithelial degeneration and at a point where the œdema and leucocytic infiltration is not very great, the number of eosinophiles is both relatively and absolutely greater than it is beneath the center of the lesion where the infiltration is denser. The eosinophiles vary from 9 to 35 in one field of the microscope (Zeiss DD objective, No. 3 eyepiece). They are scattered diffusely, but occur in greater numbers around and in the dilated capillaries. (For exudative changes see Pls. I and II, figs. 1, 2, 3.) At no point is there any evidence of the perivascular infiltration with mononuclears, which is so characteristic of syphilis.

(*c*) Regenerative changes. There is indication of slight new formation of capillaries in the corium and of a minor degree of connective tissue new growth, but the most obvious changes occur in the epithelium. At the edges of the ulcer there is a marked increase in the thickness of epithelium and a striking increase in the number and size of the epithelial downgrowths. Irregularities and distortions in the epithelial downgrowths are also seen beneath the surface of the ulcer, from which the epithelial covering has been lost. (See Pl. I, figs. 1 *d;* 2.) It is evident that, while new growth of epithelium is occurring from the columnar layer, degenerative changes are taking place in the older, more centrally placed portions of the epithelial downgrowths. (See Pls. I, II, and III, figs. 1 *e;* 3 *a;* 4.)

Tissues from these cases were treated by the method of Levaditi, with negative results, but through the courtesy of Drs. Ashburn and Craig I have had an opportunity to examine a successful silver stain of a yaws papule prepared in Washington from tissue obtained in the Philippines. In this section enormous numbers of spirochætæ are found in the degenerating central parts of the epithelial downgrowths. The spirochætæ are free in the necrotic material resulting from breaking down of epithelial cells. None were found within cells, within nuclei, nor in any part of the corium.

SUMMARY OF FINDINGS.

We may conclude from a study of these specimens, that we are dealing with a primary degenerative change resembling colliquative necrosis, affecting the epithelial structures and caused by spirochætæ, which are very abundant in the necrotic material, at least at some stage of the disease. The degeneration leads to ulcer formation. Following the degeneration there is irregular, new formation of epithelium in the form of downgrowths, which in turn often degenerate. Accompanying these changes vascular dilatation, œdema and leucocytic infiltration occur in the corium, with a minor degree of new formation of capillaries and connective tissue. There is no endarteritis, nor are there any other changes suggestive of syphilis. The majority of the infiltrating cells are polymorphonuclears at an early stage of the lesion, while mononuclears, many of which are of the plasma cell type, are almost equally abundant. In the tissue from the human being the plasma cells outnumber the polymorphonuclears. Polymorphonuclear eosinophiles are abundant and have a peculiar distribution.

The changes are essentially the same in lesions from monkeys and human beings.

The histological characteristics of the yaws nodules have been studied by Charlouis, Unna, Glogner, Plehn and others.

Charlouis (*Vierteljahresschrift (Archiv) f. Dermatologie und Syphilis* (1881), 431, quoted by Unna and others) describes particularly the epithelial overgrowth and the leucocytic infiltration, the hair follicles escaping, while the sebaceous glands are enlarged. Glogner (*Virchow's Archiv.*, (1902), **168,** 443) notes in addition a reduction in the connective and elastic tissue fibrils in the large nodules, and the occurrence of occasional pigment cells and free erythrocytes in the tissues. He did not find plasma cells but found mast cells and a few giant cells. He also encountered a lymphocytosis in the blood of 30 to 50 per cent of the patients. Plehn (Mense, Handbuch der Tropenkrankheiten (1906), **2,** 60) states that the hair follicles and sweat glands are unaffected, that the cutis is infiltrated with cells, chiefly plasma cells, and that the nodule presents the histologic picture of a granuloma, which can be distinguished from syphilis (1) by the fact that in yaws the primary affection is in the epithelium, in syphilis it is in the cutis, (2) in yaws the œdema is greater and there is no periarteritis nor endarteritis, (3) the infiltrating cells are of a different character in yaws, and there are no signs of necrosis in this disease. He found no giant cells.

The most detailed description is that given by Unna. (*Die Histopathologie der Hautkrankheiten.* Orth's Pathologische Anatomie, Berlin (1894), Ergänzungsband **2,** 503.) He quotes Pontoppidan (*Yaws und Framboesia* (1882), 201) as stating that the primary seat of the affection is in the prickle cell layer of the epithelium. Unna's description is based upon the study of a large, full-grown dry yaws nodule. The nodule rose abruptly from the level of the skin. The epithelial overgrowth began at the border and rapidly increased in extent within the limits of the nodule. The papillæ were not more numerous, but were ten or twenty times as long as usual. There was marked hyperkeratosis with accumulations of a many-layered, horny covering. Acanthosis led here, as in condyloma, to the formation of prickle cell masses, principally as interpapillary projections, while the superficial layer of prickle cells was reduced, and occasional, minute hæmorrhages escaped through into the crust above. Epithelial mitoses were infrequent. At the periphery, the granular layer was reduced, with crust formation from fibrin and leucocytes, at other places the granular layer was increased in thickness. Fibrin and leucocytes were found also between the deeper epithelial cells. The chief change in the cutis was a solid, cellular infiltration consisting chiefly of beautiful, large plasma cells. These surrounded like a mantle the epithelial projections and spread in thin lines into the separate papillæ. Beneath the epithelial growths they spread out into a uniform layer with processes extending laterally and downwards. The processes accompanied particularly the greatly dilated veins and were not directly related to the epithelial structures. The lumina of the sweat glands were dilated, and the epithelial lining swollen, while the hair follicles and hair shafts were unaltered either in the deeper tissue, where the plasma cell accumulation occurred, or while penetrating the acanthomatous part at the surface. At no place was there evidence of plasma cell degeneration, the tumor representing the purest type of plasmoma tissue.

The spindle cells of the cutis were enlarged, but not appreciably increased in numbers. Except for the usual rarefaction around the plasmoma foci, there was no progressive nor regressive change in the interstitial collaginous and elastic tissue. There was no appreciable increase in mast cells.

The pigment, which was heaped up in the basal prickle cells at the periphery of the nodule, spread in streams between the cells of the hypertrophied epithelial portion. The streams surrounded nuclei and entire, unaltered epithelial cells, producing appearances like pigment cells. There were other "pigment cells" of the same structure with two or three nuclei according to the number of epithelial cells which were surrounded by the pigment stream.

"All in all the structure of the frombœsial nodule is simple; a marked epithelial growth with hyperkeratosis accompanied by plasmoma formation in the cutis.

The absence of any degenerative changes in the plasmoma, either as giant cell formation or as fusion, makes the structure simpler than that of a syphilide of which the yaws nodule is otherwise suggestive." It is especially like a condyloma, from which it is distinguished by the greater dryness of the cutis in yaws and by the more marked keratinization. This explains the firmness and resistance of the nodule. Its cranberry form results from the overgrown papillary bodies which are covered by such a thin layer of prickle cells—that is, it is the result of the great vascular dilatation in the papillæ.

He thinks Charlouis was mistaken in interpreting his infiltrating cells as leucocytes, and suggests that they must have been plasma cells, while the leucocytes entered as a result of secondary infections. He thinks that "there is no question that the cause of frambœsia should be sought only in the first stages of the exanthem and should be looked for in the cutis." He observed the "abscess-like areas" in the keratinizing epithelium, but attributed them to secondary pyogenic infections. As another point against Charlouis' interpretation of the leucocytes he mentions that he could not find them around the dilated capillaries or veins.

Comparing these accounts, is seems clear that the histologic picture of the yaws papule at an early stage is somewhat different from that of the older nodules. The description given in this article and those given by Charlouis, Glogner, and to a less extent by Plehn, evidently refer to the younger nodules, while Unna's description is true only of the older ones.

The characteristic features of the early stage are (1) the epithelial degeneration, with (2) epithelial downgrowths into the cutis in the form of irregular columns; it seems clear that this appearance is due to the actual downgrowth of epithelium and not entirely to the upgrowth from the cutis, (3) capillary dilatation with engorgement, marked œdema and cellular infiltration limited quite sharply to the cutis and most marked at the under border of epithelium. The cells occurring are chiefly polymorphonuclears, large and small mononuclears, and plasma cells and œsinophiles. It is clear that the infiltrating cells are derived, at least in great measure, from the vessels. In addition Plehn found mast cells and Glogner giant cells. This last finding has been verified by no other writer. The changes in the fixed tissue cells of the cutis are relatively slight.

In the older nodule the chief difference concerns the infiltrating cells. The epithelial changes are the same, the œdema has largely disappeared, and the plasma cells are present in such enormous numbers as to dominate the picture. Here again, the changes in the fixed tissue cells of the cutis are of minor extent.

Remembering the remarkable regenerative power of epithelium, and noting how slight are the degenerative and regenerative changes in the cutis, we can understand how it comes about that when recovery occurs there is so little scar formation at the seat of a yaws ulcer.

A comparison of the descriptions of the different writers mentioned above, with Unna's description of the syphilitic condyloma does not leave a wide margin of difference. The differences are that the degeneration in yaws is confined to the epithelium, the spirochætæ being found in the degenerating areas, while the changes in the cutis are unimportant; that there is no periarterial or endarterial change in yaws; that the infiltrating cells at the early stage of the nodule are different from those in syphilis; and that there are no areas of necrosis and no giant cells in yaws, with the exception of the cases described by Glogner. The clinical appearance of yaws is so characteristic that it is surprising to find how closely the histological description agrees with that of the syphilide. It will be important to make a comparative study of the yaws papule and condyloma, examining lesions of the same ages, and using the silver impregnation method in the demonstration of the parasites. The claim is made that syphilis attacks primarily the cutis, while yaws, as we have seen above, is essentially a disease of the epithelium.[1]

---

[1] In an article which has recently arrived, Schüffner (*München. med. Wchnsch.* (1907), **54**, 1364) reviews one hundred and twenty-nine cases of yaws seen in Sumatra, and for the sake of completeness his article is abstracted. In one hundred and four cases he found the treponema, and of those cases examined more than once he found it in 98 per cent. The Romanowsky stain, or some modification, proved most satisfactory, especially when preceded by osmic acid or formalin vapor fixation. By the use of Levaditi's silver stain he found that the parasites occurred only in the diseased portion of the epidermis, especially in the deeper layers of prickle cells, and in this situation they were often extremely abundant. They were entirely absent elsewhere, notably in the perithelial situation common in syphilis.

While syphilis and yaws are closely parallel, he is convinced that yaws is an independent affection.

He gives a brief review of the histologic appearance, but of particular interest is his careful description of unusual skin manifestations in yaws. Under this heading he describes ring-shaped or kidney-shaped efflorescences and others which are impetiginous or vesicular. In others there was a definite roseola. In more than one-fourth of his cases there was a peculiar, macular eruption in which rounded spots from 1 to 3 centimeters in diameter were surrounded by minute papules, often becoming vesicular. Of especial interest is his description of the bone and joint pains in yaws, which he found to occur in 20 per cent of cases in adults. Periostitis was also very common. He thinks from his studies of yaws that as the result of further investigation and discrimination "syphilis will be dissolved into a group of independent diseases."

ILLUSTRATIONS.

PLATE I.

FIG. 1. Young papule from patient with leprosy and yaws. Magnification, 95 × 1. *a*, Edge of crust over the ulcer, composed of necrotic epithelium, leucocytes, etc. *b*, Hypertrophied epithelium. *c*, Epithelial downgrowth, with leucocytic infiltration. *d*, Base of ulcer, showing preservation of deepest layer of epithelium, beginning epithelial downgrowth, and covering of epithelial débris. *e*, Downgrowth, showing preservation of the columnar cell layer with degeneration of the central portion. *f, f*, Capillary dilatation in the œdematous cutis.

2. Early papule from monkey. Magnification, 95 × 1. *a*, Beginning loss of surface epithelium. *b, b*, Epithelial overgrowth. *c*, Cross section of epithelial downgrowth, showing central degeneration. *d, d, d*, Capillary dilatation in œdematous corium. *e*, Portion enlarged in Pl. II, fig. 3.

PLATE II.

FIG. 3. Portion of fig. 2 enlarged. Magnification, 415 × 1. *a*, Beginning degeneration of epithelium. *b, b, b*, Capillary dilatation.

PLATE III.

FIG. 4. Cross section through an epithelial downgrowth from an older nodule from a monkey, showing the necrosis of the central cells with preservation of younger cells at the margins. The œdema of the cutis also is well indicated. Magnification, 520 × 1.

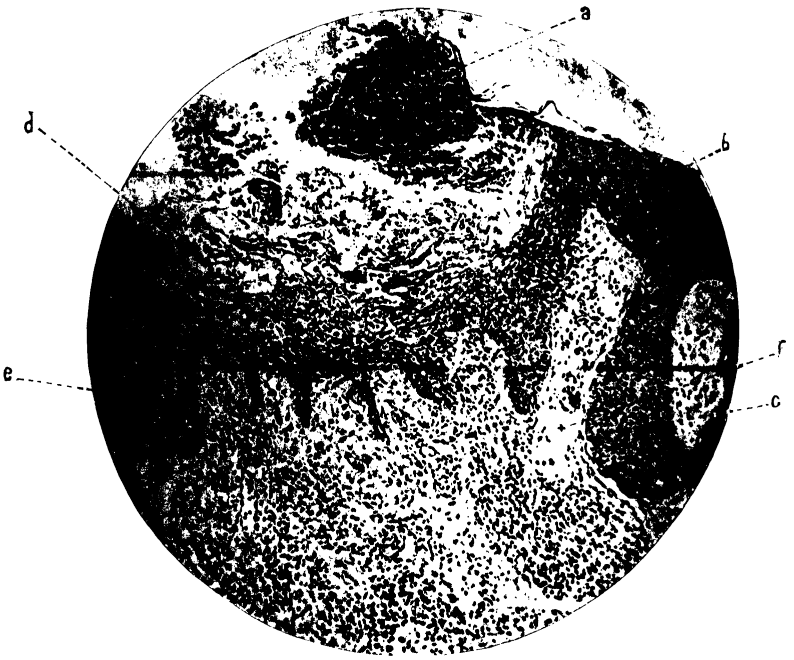

FIG. 1.

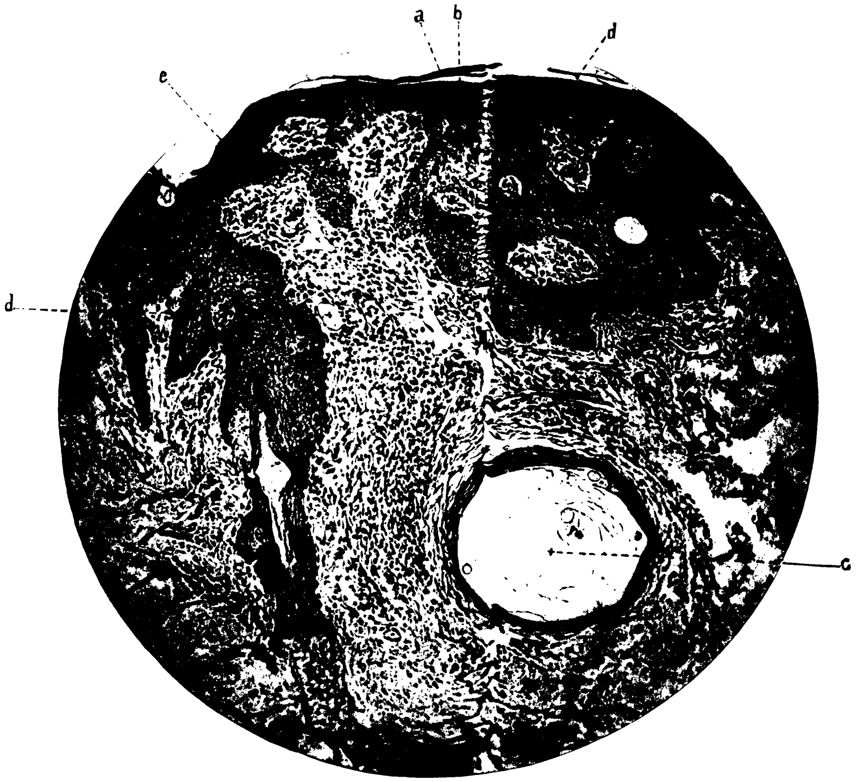

FIG. 2.

PLATE I.

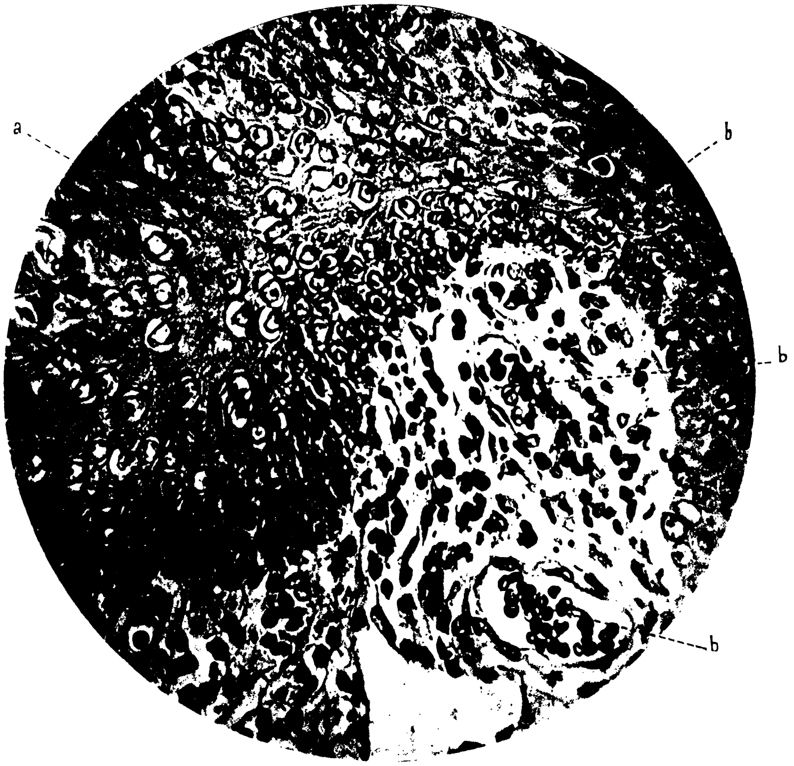

FIG. 3.

PLATE II.

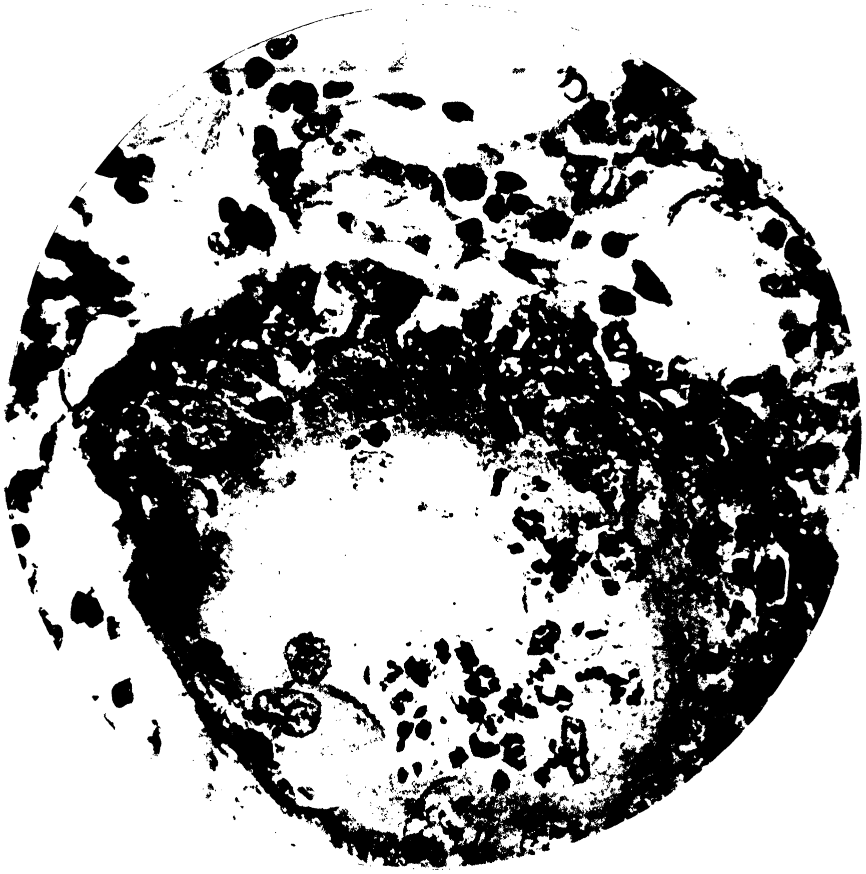

FIG. 4.

PLATE III.

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THE ETIOLOGY OF MYCETOMA.

REPORT OF A CASE OF THE OCHROID VARIETY OCCURRING IN THE PHILIPPINE ISLANDS AND CAUSED BY A NEW SPECIES OF STREPTOTHRIX (*STREPTOTHRIX FREERI*).

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OUTLINE.

- INTRODUCTION.
- CASE REPORT.
  - Study of the amputated foot.
    - Gross pathology.
    - Histology.
- THE STREPTOTHRIX.
  - Morphology.
  - Cultures.
- ANIMAL EXPERIMENTS.
- HISTORY AND LITERATURE.
- DISCUSSION AND CONCLUSIONS.
- BIBLIOGRAPHY.

INTRODUCTION.

Fully to appreciate the confusion which exists at the present time concerning the etiology of mycetoma, it is only necessary to consult the two systems of medicine which are now appearing simultaneously in the English language. J. H. Wright, in Osler's Modern Medicine, maintains that the black variety of mycetoma is a separate and distinct disease caused by a *Hyphomycete* which he cultivated from the black granules taken from a patient encountered in the Massachusetts General Hospital, although the author states that the etiologic relation of the *Hyphomycete* remains to be established by animal experiment. Wright, in discussing the ochroid variety of the disease, says that "at the present time the ochroid or pale form of mycetoma must be regarded as actinomycosis of the part."

On the other hand, MacLeod, in Allbutt's System of Medicine, recognizes Vincent's *Streptothrix maduræ* as the cause of the ochroid variety and states that it is a *Streptothrix* closely allied to, but differing from *Actinomyces.* This author, referring to the black type, is of the opinion that the fungus is a degenerate variety of the one causing the ochroid form of infection, rather than that it is a distinct species. MacLeod therefore considers the two varieties of the disease as being different stages or types of the same infection and of a single etiology. Other recent observers believe that the condition may be the result of the action of several closely allied species of *Streptothrix.*

We have isolated by culture and demonstrated conclusively the etiologic rôle of a new species of *Streptothrix* in a case of the ochroid variety of the disease, as will be seen in the following report, and have written to various colleagues for cultures of other organisms which have been isolated from types of the disease. As soon as practicable we hope, by a comparative study of these cultures and by observing their action on monkeys, to determine the etiologic importance of the other cultivated species of the *Streptothrix.*

CASE REPORT.

Mycetoma, ochroid variety; duration three years; amputation of foot; recovery. Streptothrix (Streptothrix freeri sp. nov.) cultivated from the lesions and its etiologic significance established.

P. B. (St. Paul's Hospital, No. 3256).—A Filipina woman from the Province of Bulacan; age 30, married, with two healthy children 2 and 5 years of age; was admitted on August 5, 1907, to Musgrave's service in St. Paul's Hospital. She complained of a large and painful right foot and stated that the disease was of three years' duration. The family and previous history of the patient furnished no data of importance. The patient stated, with reference to the present disease, that about three years before admission, while she was doing laundry work in the river, a sliver of bamboo entered the flesh between the great and second toes of the right foot. The splinter was withdrawn and the wound then healed. After about one month, pain in the region of the injury developed and the wound again opened and discharged pus. Following the first lesion, other nodular lesions developed in various places in the foot, and then subsequently opened and discharged pus. The foot slowly grew larger until it attained its present size.

The patient, on admission, is a somewhat frail, anæmic woman, apparently about 40 years of age, although she states she is but 30. She says she is in good health, were it not for pain and soreness of the right foot and her inability to walk, because of the size and weight of this member. The heart and vessels, if a considerable prominence of the superficial veins about the chest is excepted, appear to be normal. The blood is free from parasites. Hæmoglobin, 85 per cent; red cells, 4,200,000; leucocytes, 7,000. The leucocytes by differential determination show 4 per cent eosinophiles as the only departure from normal. Her appetite is good and no disturbance of the bowel function is apparent, although the cæcum on palpation appears to be thickened and tender. There is no cough, and a physical examination of the chest is negative. There are no symptoms attributable to disturbance of the urinary system and the urine is normal. The uterus is retroflexed and bound down by adhesions and there is tenderness in the region of the right ovary. The cutaneous system appears to be normal, excepting over the right foot. The superficial lymphatics, particularly in the right groin, are moderately enlarged and quite firm. Pain of a dull character and not severe is present in the right foot, more pronounced when the patient is in the upright position. The reflexes and sensation in general are normal.

The right foot (shown in Pl. I, fig. 1) is much enlarged and has the appearance described in mycetoma. The toes are spread apart and appear to be turned upward, because of the swelling of the ball of the foot. There are numerous punched-out, craterlike openings which communicate by sinuses throughout the foot. These are so extensive that a probe may be passed in almost any direction through the member. Pressure shows a moderately firm, but spongy consistency and if applied on one point will cause a discharge from several openings at the same time. There is some general tenderness and but slight alteration in sensation. The process apparently stops short at the ankle. Other, earlier lesions are present in addition to those already mentioned. These appear somewhat to differ from the usually described, early lesions of mycetoma. They consist of soft, dark-colored, nodular masses of various sizes which, when opened, bleed most profusely, exuding a dark venous-like blood.

The lesions, when examined microscopically, in addition to blood, contain other degenerating cells. The *Streptothrix* about to be described may also be observed. Cultures and smears were made from the lesions. The *Streptothrix* was seen in the smears, but all cultures made at this time remained sterile.

The patient was transferred to the surgical service of Drs. McDill and Dudley, and acting upon the recommendations made in the literature, Dr. F. W. Dudley performed an amputation of the leg at the lower third.

The patient made a satisfactory recovery from the operation, and after five months there has been no recurrence of the disease.

STUDY OF THE AMPUTATED FOOT.

Gross pathology.—The foot was sent to the authors at the Bureau of Science immediately after the operation. An examination of sawed sections of the tibia and fibula shows that the mycetomatous lesion extends to about 5 centimeters above the ankle joint, the parasitic growth being demonstrated in the bone marrow and the medullary canal of the tibia. The appearance of the foot in general corresponds with the usual descriptions given for a well-advanced mycetoma of the ochroid variety. There is general enlargement to at least twice the normal size. This enlargement is perhaps more marked on the ball of the foot, giving to the toes the appearance of being turned upward and spread apart. The external lesions are of two kinds, the first being craterlike, nodular and more or less whitish in color, usually containing an opening in the center; the second are the earlier, reddish, soft, fluctuating nodules, which when opened seem to contain a dark material appearing like venous blood. Stages of the lesions in character between these two may be seen, and smaller nodules, from 2 to 8 millimeters in diameter, resembling the large ones in all respects except that they contain no openings, are also present. The skin in general is not much thickened, but it is rather tense over the swollen subcutaneous and other tissues. The openings on the cutaneous surface are directly continuous with sinuses which run in all directions through the foot and which connect freely with each other. It is very easy to section the foot, the knife passing readily in all directions because of almost complete destruction of the bony tissues. The cut surface shows a mass of disorganized and degenerated tissue, *without evidence of inflammation,* and an abundance of oily, slightly tenacious liquid exudes not only from these surfaces but also from the sinuses exposed by the section. This fluid contains the so-called "fish roe" granules, which vary in size from those only observable under the microscope to others 2 millimeters or more in diameter. The masses have a yellowish color and when pressed out and examined under the microscope are seen to be made up of degenerated cells, débris, and the *Streptothrix* about to be described.

Histology.—Nothing very definite can be observed in the sections. There is uniform destruction of the tissues, with but slight evidence of repair. Many specimens from all parts of the diseased foot were removed for section and were prepared by the Zenker, alcohol and formalin, and by the Levaditi method; and for purposes of study many stains were used, including the Ziehl-Neelson stain for tubercle bacilli; the Gram-Weigert; Levaditi's methylene blue; Wright's blood stain; Giemsa's and the general aniline dyes. The first two of these were very satisfactory, particularly the Gram-Weigert method.

The following method used by Mr. Willyoung is particularly good for studying sections of the larger granules:

> The tissue is stained in warm carbol-fuchsin for ten minutes, decolorized in 5 per cent nitric acid-alcohol for from five to fifteen seconds; washed in water, counter-stained for five minutes in a concentrated aqueous solution of methylene blue; washed in water, then in 95 per cent alcohol and finally in absolute alcohol, cleared in xylol and mounted in balsam. With this stain the *Streptothrix* takes a bright red color, whereas the tissues become blue.

Sections taken from the skin at a distance from the openings demonstrate very little change. In some places the walls of the small blood vessels are thickened and in a few instances these are surrounded by an area of moderate, round-cell infiltration. Sections through the margins of the openings show a necrotic zone without any areas of infiltration, or any other marked evidence of tissue repair or reaction. Some of these sections contain small granules of the *Streptothrix* and in other instances mycelial threads may be seen. The alteration in tissue in sections from many other places in the foot is so great as to render the differentiation of the various elements impossible.

The whole foot is practically a mass of degenerated tissue, débris, and fungus elements, in which an occasional, recognizable piece of nerve, bone, or tendon may be seen. There are areas of fixed tissue-cell proliferation, in places usually surrounding a partially occluded blood vessel or a granule of the fungus mass. Evidences of repair of tissue or of acute inflammation are almost entirely absent. The *Streptothrix* is found in the sections in the form of granules and of occasional mycelial threads, both of these types being evident in masses of débris lying in somewhat definitely outlined cavities, and again among better preserved tissue cells. The granules vary in size from about 25 μ to 5 millimeters or more, and although they usually are oval or round in the smaller masses, they are of dumb-bell or more irregular shapes in the larger ones. The smaller granules, when appropriately stained, appear as conglomerate masses of mycelia, like puffballs, in which degenerated cells, crystals, and unstainable débris may be embedded; the margins of these granules often show a more or less definite radiation of fibers from the central mass, coccoid and other irregular forms being rare. The picture is somewhat different in the larger granules. These, when they are cut through in the process of sectioning, show three fairly well-marked zones. The outer of these is made up largely of radiating, mycelial bodies of varying shapes, some appearing as hyphæ, others as twisted and bent mycelia, and in some sections a more or less definite "club" formation may be noted. The middle zone is composed of irregularly interwoven mycelia with a few irregular forms, a mass of poorly staining material, degenerated cells, and a few crystals is embedded in it. The third or inner zone seems to resemble a cavity filled with granular and hyaline matter, broken-down cells and irregular forms of the *Streptothrix*; these latter being of several shapes, of which the bacillary and coccus-like predominate. (Pl. 3, fig. 1.)

THE STREPTOTHRIX.

Morphology.—A *Streptothrix* answering to the following description was found in large numbers in the discharges from the open sinuses and the various tissues of the diseased foot after amputation; it was cultivated and the disease was produced in monkeys' feet by inoculation with cultures and scrapings from the amputated member.

The discharges and scrapings from the cut surfaces of the foot, on microscopic examination, show the distribution of the *Streptothrix* to be very general and not confined to any particular tissue, or even to the sinuses and cavities. The organism is easily recognizable in fresh coverglass preparations. It occurs in several forms. The most important of these are granules of varying sizes, the larger ones being of the "fish roe" type discussed by several authors. These granules are of a dull, yellowish-white; the yellowish color being more pronounced in the larger and hardly noticeable in the smaller ones. They are of a tough, dough-like consistency, they may be washed and handled with impunity, and show evidences of considerable elasticity. They vary in diameter from 0.25 to 5 millimeters or more, are of diverse shapes, the very small ones usually being oval, the conformation differing as they grow larger. When pressed out by a cover glass and examined under a low power of the microscope, they somewhat resemble in appearance similar preparations of *Actinomyces hominis*; however, the ray formation is a much less distinctive feature and the "club" appearances are absent, or much less noticeable. Under the high powers of the microscope the smaller granules are seen to be made up of a mass of interwoven, fungoid filaments with a more or less definite picture of radiation of the filaments at the margins. The central portion in larger and older granules contains fewer of the filamentous forms, but there are numerous, bacillary and coccus-like varieties together with crystals, bright, shining, small, round bodies, and débris, the whole making up a more or less dense mass which, as it were, constitutes a cave for the granule. Outside of this mass there is a zone of interlacing filaments, in which cells and débris are embedded and, finally, the outer fringe is made up largely of terminal branches, loops, and hyphæ, which are forms of the fungus. Here, as is the case in the smaller nodules, there is some attempt at radiation and in certain of the specimens definite "clubs" may be seen. The *Streptothrix* likewise often appears in the fresh material as a somewhat skein-like mass of filaments or mycelia, with hyphæ and occasional round forms. These filaments are often found free in the discharge, and because of their loose structure are good specimens for examination. Still other types of the *Streptothrix* are sometimes present. These consist of a single filament, or of a few small, round, glistening bodies lying free in the secretion. The nature of these small, round, refractive bodies is not entirely clear. They are found free, bound up in meshes of mycelia, and what appear to be similar structures are sometimes observed inclosed within large fixed tissue cells, this process of inclosure apparently being one of phagocytosis. Yellowish crystals of various sizes and shapes, the products of parasitic activity, occur in the discharge in small groups, or singly. A pigment of the appearance of hæmoglobin may also be seen.

The individual elements of the *Streptothrix* give interesting pictures when they are examined with the highest powers of the microscope. The hyphæ-like forms seem to be made up of a delicate membrane, covering a homogeneous and unbroken core; in the older, mycelial types the outer membrane appears to be irregular in thickness and density and absent in places; the inner portion is also frequently and irregularly broken, and thus the appearances of bacillary and ovoid or coccal sections is produced. Branching forms are plentiful, the branches first apparently appearing as buds, which grow to hyphæ from the side of the mycelial thread. True dichotomous division has not been observed. The small, round bodies mentioned above often appear to have a double containing wall and they vary in diameter from 3 to 10 μ.

Staining.—The *Streptothrix* takes the majority of the ordinary stains in preparations of fresh material; it is stained by the Gram-Weigert method, and its acid-fast and alcohol-fast properties are quite marked, particularly in the older filaments.

The Ziehl-Neelson-Gabbet method for staining the tubercle bacillus is also very satisfactory for *Streptothrix freeri*, the fungus elements assuming the same bright red color which is imparted to tubercle bacilli. The structure of the granules becomes fairly apparent by the various methods of staining and the specimens confirm the observations already recorded when discussing the fresh materials.

Stained specimens which show the individual elements give some interesting results. Some specimens, when they are colored by the method used for tubercle bacilli, contain so many of the bacillary forms which take the red stain and which closely resemble *Bacillus tuberculosis*, that we at first thought the latter organism was also present in our cultures. However, further study has shown these, as well as the coccal and other irregular forms, to result from the breaking up of the older mycelial filaments. These bodies which resemble tubercle bacilli may sometimes be seen in the filamentous mycelia, still surrounded by the unbroken sheath.

Cultures.—Cultures on agar and glycerin agar, from the open wounds before amputation and after the foot had been dressed for twenty-four hours in a wet bichloride dressing, remained sterile. The *Streptothrix* was cultivated without difficulty from the sinuses and tissues of the amputated foot and no bacteria were present except when the cultures were taken from those surfaces contaminated by the knife in opening the foot; in the latter instances saprophytic bacteria were also present.

Lesions from which the *Streptothrix* was obtained in pure culture were produced in monkeys after the inoculation of fresh material. The microörganism is aërobic and it grows with greater or less readiness upon most of the ordinary laboratory media. Cultures upon media containing sugar or glycerin and upon potato have some color, which varies in intensity.

A profuse growth appears in a few days in certain media, but in others the development is much slower. Potato, sugar containing media, and glycerin agar at 37° C., are best adapted to the growth of the fungus, but a slower development takes place even at ordinary room temperature (30° C.). The organism is an obligate aërobe.

Potato.—Growth becomes visible after twenty-four hours and after seventy-two becomes luxuriant. The colonies, when they first become apparent, are small, discrete and slightly raised, and have a delicate, pink color. As growth proceeds they become larger at the base and raised to a height of at least 0.1 millimeter. The upper portion of the colony appears as a short shred which often folds upon itself, giving it the appearance of being umbilicated. The whole inoculated surface finally changes to a heaped-up mass which has a yellow-ocher color in the center, with a pinkish or white periphery. The medium becomes darkened and moist. The growth may be lifted in heaps on a platinum loop. Desegmentation occurs when it is placed in water and small, flat particles, from delicate pink to a yellowish-brown color and which resemble dry bran, float upon the surface; on shaking the vessel the particles adhere to the sides of the vessel. (Pl. IV, fig. 2.)

Glycerin agar.—Growth appears after three days; later it becomes heaped up, the medium remaining moist. The colonies at first are discrete, but they soon become confluent, but not diffuse; they are raised and in some instances have the umbilicated appearance observed on potato. As the cultures grow older, they to some extent resemble those of the tubercle bacillus on the same medium. A yellowish pigment is produced, more pronounced in the central portion where the growth is more luxuriant; with a pink to pinkish-white periphery. The growth may readily be peeled from the surface of the medium, and when observed in bulk it has a moist appearance. No pigmentation of the medium occurs. (Pl. IV, fig. 1.)

Agar-agar 1 per cent acid and 1 per cent alkaline to phenolphthalein.—The growth after four days' incubation appears as a smooth, white and glistening, very slightly raised mass. The colonies do not develop the central pit, thus differing from their appearance on potato, glycerin agar and the media containing sugar, neither do they produce the pigment which is characteristic of growth on the latter, but on the contrary they first assume a porcelain-white color and later develop a delicate, diffuse pink. There is no pigmentation of the medium.

Loeffler's blood serum.—Growth on this is much slower than on other media. The pigmentation and growth are very similar to those on plain agar-agar. After two weeks' incubation no change in the medium occurs.

Glucose agar stab.—Colonies do not develop along the track of the needle, but they grow luxuriantly on the surface of the medium forming a heaped-up center, with a wrinkled periphery. The center is yellow in color, the periphery pink to pinkish-white. No pigmentation of the medium occurs. The growth on this medium shows the aërobic tendencies of the microörganism.

Gelatin.—This medium is not liquefied; growth is similar to that on glucose agar.

Bouillon, alkaline and acid.—Floating, flat particles appear on the surface of the medium after three days' incubation. These particles, when the tube is shaken, adhere to its sides. On further incubation, a sediment in the form of a tenacious mass settles to the bottom. The medium does not become cloudy, but on long incubation it changes to a darker color.

Alkaline litmus-milk.—Growth appears after three days on the surface of the medium in the form of dry, flat particles, which later become confluent, forming a heaped-up, yellowish mass. A tenacious sediment forms at the bottom of the tube; in this portion of the medium the milk is gradually decolorized; however, it does not become red. The milk is not coagulated.

Lactose-litmus agar stab.—The growth on this medium is similar to that on the glucose-agar stab and it produces no change in the reaction of the medium.

Potato infusion.—The growth is similar to that on bouillon; however, no pigment is produced and the medium is not altered.

Relation of growth to temperature and resistance to heat.—Growth was observed both at room temperature and at 37° C., but under the former condition it was much slower and less pigment is produced than at the temperature of the incubator. No growth occurs in cultures after exposure to 70° for 15 minutes.

ANIMAL EXPERIMENTS.

The series includes forty monkeys (*Cynomolgus philippinensis* Geoff.), guinea pigs, rabbits, dogs and pigeons. In nearly every instance lesions characteristic of mycetoma were artificially produced by intraperitoneal inoculation, with the exception of the rabbits and pigeons. Three typical examples of Madura foot developed in monkeys after injection of the organism into the foot. (See Pl. I, figs. 2 and 3.) On the other hand, in no instance was a progressive disease produced by *subcutaneous* inoculation in other parts of the body.

INTRAPERITONEAL INOCULATIONS OF MONKEYS.

Monkey number 3268.—Inoculated into the abdominal cavity with material from the original case. After five days, a small tumor was located above the bladder in the left inguinal region and three days later the animal was found to be in a dying condition. It was immediately killed and autopsy performed.

Autopsy.—Heart and lungs appear normal; no fluid in either cavity. Spleen, liver and kidneys normal, some fluid found in the abdominal cavity. A tumor 2 centimeters in diameter is present just above and to the left of the bladder. This tumor is surrounded by adhesions and is adherent to the omentum, abdominal wall and intestine, it is extremely firm in the center, with a surrounding œdematous coat. The cut section shows several small sinuses, from which small quantities of pus may be expressed. Small, distinct, refractive granules can be seen macroscopically, none larger than a pin head. Smears show the presence of a *Streptothrix* and *S. freeri* was reclaimed in pure cultures.

Monkey number 3269.—Inoculated with the material from the previous animal. After six days the animal showed a disposition to sit in a cramped position. On palpation a nodule, about 2 centimeters in diameter, which seemed to be attached to the abdominal wall was located in the abdominal cavity. Eight days later the animal was found to be in a dying condition. It was killed and autopsy performed immediately.

Autopsy.—Lungs, heart, liver and kidneys normal. Some fluid is found in the abdominal cavity. Above the bladder, and occupying the entire abdominal cavity there is a firm mass composed of necrotic tissue and compact adhesions of the intestinal coils to the abdominal wall and to the omentum. A cut section shows numerous sinuses surrounded by thickened walls, in some instances these sinuses have burrowed through the walls of the intestines and in others into the wall of the abdomen. A thick, cream-like discharge in which small, glistening grains or granules are visible, can be pressed from the cavities. Pure cultures of the *Streptothrix* were obtained.

Monkey number 3307.—Inoculated with 0.2 of a potato slant culture obtained from monkey number 3268. After ten days a small nodule could be felt above the bladder in the left inguinal region. Several days later, multiple nodules developed, and ten days afterward the animal died.

Autopsy.—Several distinct nodules, which contain thick, cream-like pus are present. Anterior to, and a little above the bladder there is a tumor 2 centimeters in diameter, bound by adhesions to the mesentery of the descending colon, to coils of the small intestine and to the abdominal wall. On section of the tumor, a heavy, cream-like pus is discharged from the center; the surrounding mass is composed of a necrotic material containing small sinuses filled with pus. Smears show numerous growths of the *Streptothrix.*

Monkey number 3308.—Inoculated with 0.25 of a potato slant culture. The animal died ten days later, the lesions being similar to those found in animal number 3307.

Monkey number 3309.—Inoculated with 0.25 of a potato slant culture in the abdominal cavity. The animal is still living, thirty days after the inoculation. On palpation, a small nodule can be distinguished in the lower portion of the abdominal cavity.

INTRAPERITONEAL INOCULATION OF GUINEA PIGS.

Guinea pig number 3313.—Inoculated with a small amount of the culture grown from monkey number 3269. The animal died seven days after the inoculation.

Autopsy.—Ten cubic centimeters of a clear fluid are found in the abdominal cavity, the heart is slightly dilated, the lungs congested, the diaphragm shows numerous, small, superficial abscesses varying in size, the largest not more than 2 millimeters in diameter. The liver is intensely congested and contains similar abscesses, and they are also found in the omentum, mesentery and spleen. Pure cultures of the microörganism were obtained from the abscesses.

Guinea pig number 3314.—Inoculated with a small amount of the culture grown from monkey number 3269. The animal died ten days later.

Autopsy demonstrates lesions identical in character with those found in guinea pig number 3313.

Guinea pig number 3323.—Inoculated with 0.5 cubic centimeter of an emulsion of material from abscesses from monkey number 3269. At the present time, one month later, the animal is still living and seems to be in good health.

INTRAPERITONEAL INOCULATIONS OF DOGS, RABBITS AND PIGEONS.

Three healthy dogs weighing about 8 kilograms each were used in these experiments. In every case lesions identical in appearance with those described in the monkeys developed.

Ten rabbits and three pigeons were inoculated with fresh material from lesions and with cultures of varying ages, but in all cases the results were negative.

SUBCUTANEOUS AND INTRAVENOUS INOCULATIONS.

Subcutaneous inoculations into a large number of rabbits, guinea pigs, dogs and monkeys all gave the same results. Small nodules developed only at the points of inoculation; these soon underwent resolution and finally healed. In many instances pure cultures of the *Streptothrix* were obtained from the discharges.

Cultures and emulsions of the fresh material were used for intravenous inoculation of rabbits, but all the results were negative.

INOCULATIONS INTO MONKEYS' FEET.

Monkey number 3266 was inoculated with fresh material, in the left foot, after incision and scraping the metatarsal bones. Sixteen days later a marked swelling of the foot developed, and after twenty days, numerous, suppurative lesions appeared between the toes, on the ball of the foot, and on the heel. The animal died of malnutrition.

Section of the foot.—A cut section of foot showed a slight necrosis of the tarsal bones and numerous sinuses, containing pus, surrounded by necrotic material extended throughout the organ. A pure culture of the microörganism was isolated from the discharge.

Monkey number 3310.—Inoculated with 0.25 of a potato slant culture in the ball of the foot. After ten days a marked swelling developed, this afterward suppurated and broke down, discharging a viscid, cream-like pus, containing a quantity of the *Streptothrix.* At present the animal is still living and shows a typical Madura foot. (See Pl. I, fig. 2.)

Monkey number 3311.—Inoculated with 0.25 of a slant potato culture in the ball of the foot, the animal is still living and presents the same characteristic lesions as does monkey number 3310. (See Pl. I, fig. 3.)

HISTORY AND LITERATURE.

Notwithstanding the inaccessibility of many of the older articles in the original, we have been able to study practically all of the important discussions of this subject, and from these, as well as from other references, Miss Polk, librarian of the Bureau, has been able to compile a fairly complete bibliography. No article of great importance, except from an historical standpoint, existed before the observations of Vandyke Carter, which extended from 1859 to 1874. The greater part of the oldest literature is in the English Army Reports from India and therefore is not available to the general public.

Kämpfer (1712), according to Scheube, was the first European physician to mention the disease, which he discussed under the name "*Perical.*" The same author states that Heynes (1806) reviewed the subject in his historic and statistical news of India. Both of these writers, and perhaps all others before 1840, confused Madura foot with elephantiasis and other independent diseases. However, to judge from the translation of some of the older native names it seems that it was considered to be something specific and definite by the natives of India. Scheube informs us that Brett (1840) was the first author to indicate that Madura foot is a specific disease.

During the ten years from 1840 to 1850, mention of the disease occurs several times; the following may be mentioned: Gill (1842) reported it from Madura; Godfrey (1844) from Bellary; Colebrook (1844) from Madras; Gunther (1844) from Coddapah. Eyre (1848) reported cases from Bellary and according to Hirsch made the first collection of the literature and he as well as Godfrey and Colebrook[1] (1850) gave a general description of the disease, the latter indicating its specific character. Scheube states that Ballingal (1855) reported one case and first described the infection in detail as being of a parasitic nature. A careful description was also given by Eyre (1860), by Collas (1861), (Hirsch) and Biddle (1862), who reported cases, and by

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[1] Military Med. Reports, Madras (1850).

Hirsch[2] (1863 and 1886), who gave a general and historical review. Coquerel (1866) published a report of one case of mycetoma. He recognized and illustrated the fungus elements present in the lesions. Hirsch also states that Minas added several cases from Hindostan to the literature, and Atchison gave several instances of the disease from Jhelm in the Punjab. According to Le Dantec, Duval, Berénger-Férand, and Carpot encountered cases which they mentioned in the literature in Senegambia, and Legrain in Algiers. Moxon and Hogg[3] note fungus-like filaments in the granules in an instance of *ochroid* mycetoma. Bristowe[4] made a study of the melanoid granules in one case. He recognized the presence of a fungus which he considered to be identical with that discovered by Carter. During[5] and Rochefort,[6] according to Scheube, also mention the disease. Hogg[7] studied a case of the melanoid type and recognized the presence of a fungus in the black granules. The patient was cut on the foot by a stone six months before the development of the symptoms. Berkeley[8] cultivated a fungus from specimens sent to him by Carter and named it *Chionyphe carteri*, but subsequently denied the etiologic significance of this parasite. Fox[9] reported cases of the disease and in discussing the etiology questioned the importance of the fungoid elements which had been described in this connection.

Lewis and Cunningham[10] after a study of the tissues obtained from a case of the infection came to the conclusion that mycetoma was "essentially a degeneration of the fatty tissues, independent of the local presence or influence of any parasites whatever."

Regardless of the previous history of mycetoma, we owe the establishment of the condition as an independent and specific disease to which this author gave the accepted name, mycetoma, to the researches of Vandyke Carter, who began the work in 1859 and who finished his publications by a monograph[11] in 1874. In the first of his articles Dr. Carter strongly upholds the fungus nature of the infection and his publication is so complete as to leave but little to add, even at this day, excepting discussions concerning the etiology and histology of Madura foot. Carter states that in October, 1859, he examined an amputated foot immediately after operation and "found the clearest possible evidence of the true fungus structure of the black (melanoid) particles now well enough known." This observation was afterwards invariably confirmed by himself and, according to his own statement, also by Bristowe.[12] This discussion concerns the *black* variety of the infection.

[2] *Arch. f. path. Anat.*, Berl. (Virchow's Arch.) (1863), **27**, 98, and Handb. der historisch-geographischen Path. (1886).

[3] *Trans. Path. Soc.* Lond. (1870).

[4] *Trans. Path. Soc.* Lond. (1871) (Vincent gives it 1881).

[5] Eulenburg's Real-Ency. d. ges. Heilk. (3d ed.) **14**, 203.

[6] *Arch. d. Med. Nav.* (1876), **25**.

[7] *Med. Times and Gaz.* (1871), July 22, 93, and *Trans. Path. Soc.* Lond. (1872), **18**, 294.

[8] *Med. Press* and *Cir.* (1876), 465.

[9] *Trans. Path. Soc.* Lond. (1870), **21**, 411, and (1871), **22**, 320; *Lancet* (1876), 190.

[10] 11th An. Rep. San. Com. Govt. India, Calcutta (1875–88).

[11] On Mycetoma or the Fungus Disease of India, Lond. (1874).

[12] *Trans. Path. Soc.* Lond. (1871), **22**.

In November, 1859, Carter examined a foot before and after amputation, and found in it and in the discharges from the openings on the surface *"numerous minute, pale particles,* which also presented a decided fungus structure." The fungus-like appearance of these bodies was subsequently confirmed, Carter says, by the members of the Medical and Physical Society to whom he showed his specimens at one of their meetings. Therefore, Carter concluded that although there were apparent differences between the fungi of the pale and black types, and that those of the former were very peculiar structures, *Chionyphe* could be seen to spring from the particles (sclerotia) obtained from them as well as from those of the *black variety,* and it was his opinion that the two forms are but varieties or different stages of the same disease.

After further discussion Carter concludes as follows:

> "The foot disease of India is not a carious, strumous, or myeloid, or any like affection; but a veritable parasitic disease, due to the growth and extension, within the tissues of the human foot, of an indigenous mould or fungus of true plant nature."

It is apparent from a review[13] of his monograph[14] that Dr. Carter was a pioneer in his observations in the establishment of the cause of the disease, and in maintaining the identity of the two varieties. However, the reviewers of his monograph criticized Carter's conclusions and pointed to the lack of evidence submitted by him in support of the parasitic nature of the so-called fungus.

Corre (1883), in reporting the notes of Dr. Collas, states that the latter observer considered *Epulis gravis,* the pseudo-cancer of the inferior maxilla found in Pondicherry, to be identical with mycetoma. Vincent, Roux (1888) and Brun (1893) describe the early symptoms of the disease; Lebroux (Bordeaux 1887) in a thesis (not available to us) discussed the whole subject of mycetoma and Bassini (1888) reported a case (the first in Europe) of melanoid mycetoma which occurred in Italy. He recognized the fungus nature of the granules, the radiating type of the filaments and the irregular swellings in segments. He considered them to be somewhat of the nature of the *Aspergilli* or *Mucorini.* Bassini's patient pricked his foot in an ox stall; the wound healed, but a tumor developed and by the seventh or eighth month the patient could not walk. The fungus was found in the typical tumor removed at operation, but attempts at cultivation were not successful.

The decade from 1890 to 1900 furnished a number of interesting contributions to the subject.

Hewlett (1892 and 1893) in his first article described findings obtained from the study of a preserved specimen sent from India and concluded that mycetoma is identical with actinomycosis. In his second paper he reports two cases of the ochroid variety, and reiterates his belief that the process is the same as actinomycosis.

Kanthack (1892 and 1893) studied sections from the prepared material obtained from three cases of the black variety; he noted the fungoid elements and although he "found it difficult to convince himself of their vegetable nature," he thought that they might be degenerating types of an organism similar to *Actinomyces*. Kanthack also examined sections from preserved material taken from twelve cases of the ochroid variety, and states that "the parasite of the ochroid variety agrees morphologically and structurally with the typical and perfect ray fungus, and there can be no reasonable doubt that both belong to the same botanical group." Kanthack concludes that "(1) the fish-roe masses are undoubtedly a form of *actinomyces;* (2) the black masses in their most perfect shape are also of this nature, but in a state of degeneration; (3) a degenerated form of the yellow variety is occasionally found, in appearance not wholly unlike these black masses, so that the relation between all these forms seems to be fully established."

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[13] *Lancet* (1874), 591.

[14] "On Mycetoma or the Fungus Disease of India, Lond." (1874), 113.

Boyce and Surveyor (1893 and 1894), in addition to a thorough discussion of the etiology of the disease with special reference to the difference between the two varieties, have given one of the most succinct and comprehensive general descriptions of mycetoma which we have seen. Their description is so lucid and brief that we quote from it as follows:

"The Madura foot of India is a very chronic affection, lasting in some cases twenty-five years. It is purely a local disease of the extremities, chiefly the foot, and generalization has not been observed. It usually occurs in people who go barefoot and are working in fields. In most cases it has been traced to some injury. The big toe is often affected at first. The disease stops at the ankle for a short time, then it spreads up to the knee, and eventually may even reach the thigh. One of us has had the opportunity of seeing a recurrence in the scar after amputation; this, however, must be very rare, and amputation affords complete relief * * * and is, indeed, one of the most successful operations in India. The foot * * * is greatly altered; it is enlarged, often many times the natural size. The overgrowth of the foot is irregular; the toes may become buried, * * * and the surface becomes studded over with mammillated, or even villous, projections. A large number of the mammillated projections mark the presence of sinuses, which pass deeply into the foot; and on section these may be seen to honeycomb it. From the opening of the sinuses a purulent or sanguinous discharge can be pressed out, and in this are found, in one series of cases, small particles of a light yellow color, which have been compared to fish roe; while, in the remaining cases, deep brown or black particles, resembling grains of gunpowder, may be seen. The disorganization of the interior of the foot becomes very complete in time; the bones undergo a rarefactive ostitis, and are ultimately absorbed, granulation tissue also invades the muscles and fat, and lends to their disappearance. Associated with the hypertrophy of the granulation tissue there may be considerable hyperplasia of the epithelium of the skin. This overgrowth gives rise to the mammillated and papillomatous projections previously referred to.

"The only difference which one at present finds, clinically and microscopically, in the numerous cases of Madura foot is the difference in the size and color of the particles. Carter termed that form of the disease in which the black particles were present the "melanoid" variety, while under the "white" or "ochroid" were grouped those cases in which the fish-roe like bodies were found. It will be understood that only those cases of Madura disease in which the particles can be demonstrated macroscopically or microscopically will be admitted as genuine. The disease appears to be confounded with scrofula and various forms of elephantiasis, in the production of which probably other parasites play an important part."

Boyce and Surveyor examined, in a most careful manner, tissues from six cases of the melanoid and from fifteen of the ochroid variety of the infection. In an addendum to their article, they mention having studied "a large number of cases of the disease" including fifteen more of the melanoid type. The authors "bring forward reasons for believing that there are at least two distinct fungi—one, a very highly organized species, the other a very delicate and lowly organized type, presenting very many of the characters of *Actinomyces*."

By the use of sodium hypochlorite as a clearing agent in the case of the black granules, and logwood as a stain, they very clearly showed the fungus nature of the granules and their illustrations are very clear. They write, while discussing the granules of the ochroid variety, that whereas in some respects these granules resemble those of actinomycosis "it would be rash to state that the white particles are always actinomycotic." Boyce and Surveyor failed to grow the parasite upon artificial media. Their article is interesting and valuable and may be commended to anyone who wishes to review the subject.

In 1894 Boyce states that the organism of the black variety is a richly growing fungus, which is rendered more visible by solutions of sodium hypochlorite; that of the white one is a more delicate organism, closely resembling that of actinomycosis. This author had secured additional data by means of cultures of the parasite. Agar tubes, inoculated by friends in India and forwarded to him, were usually contaminated by other organisms, but one tube which contained the white variety was, upon its receipt by the author, free from them.

Microscopic examination of this culture showed colonies of a thick mycelium, firm and difficult to tear apart; the mycelium contained vacuoles, and its membrane was very thin. Club-shaped forms were not found and dichotomous branching was present. Subcultures were secured on grape sugar, agar and glycerin agar at 35° to 37°. Growth was very slow, much more so than in the case of *Actinomyces*. Colonies having a peculiar appearance slowly developed. These colonies had odd markings. At first quadrates appeared, following them there were more divisions, until finally they were separated into many segments, no fusion of the colonies being apparent. Growth is almost entirely inhibited on potato or on acid or alkaline bouillon. There was no formation of pigment on potato or agar, the growth therefore differed from *Actinomyces*. A comparison between the cultures and particles taken directly from the foot showed great similarity.

The author conducted a number of experiments with animals and demonstrated that subcutaneous inoculations of rabbits, guinea pigs, monkeys and rats always gave a local reaction which took the form of a tumor, increasing in size during two or three weeks, after which time it became smaller and harder. Boyce concluded that the fungus which he described was a new variety causing the white type of Madura foot. Boyce and Surveyor (1894) in a third article, continue their discussion of the cultivation of the parasite of Madura foot. Their material was inoculated into culture media in India and forwarded to them in England. The following quotation from their later work has an important bearing on the history of the subject:

"This organism grows slowly on most of the nutrient media we have tried. In gelatin and broth it does not seem to grow at all, but cultures taken from these media begin to grow when transferred to potato or glycerin-sugar-agar. On these media the growth does not appear to make any progress for about a week, but after that the margin around the inoculated area shows slight projections, which thicken gradually and give the growth a radiate appearance. Minute colonies are found around the central growth, and these show a cross-like depression, which becomes more marked as they increase in size. The growth is firmly adherent to the nutrient medium at the margins, while the central part is loosely attached. The color is dull white or slightly pinkish."

"The culture is brittle and crumbles easily. It grows best at 39°. It is aërobic. "In an atmosphere of hydrogen the growth is excessively slow. In this respect it differs from actinomyces which grows easily in an atmosphere of hydrogen."

The organism was readily stained by aniline dyes and by Gram's method. The filaments were long and slender, no septa could be distinguished and dichotamous division was not observed. Inoculation experiments with the cultures were not made.

Le Dantec (1894) reported cases from Africa. According to Scheube, Le Dantec cultivated *Staphylococci, Streptococci,* and short bacilli from the truffle-shaped bodies. In bouillon the bacilli grew into long filaments without ramification. Cultures on solid media were only obtained by transplants from bouillon and not directly from the material. All cultures gradually assumed a reddish-rust color and rabbits and guinea pigs were not susceptible to infection by them.

Hatch and Childe (1894) report a case of the black, or a combined black and ochroid variety, in an African negro. The disease primarily involved the knee, and subsequently extended to the abdominal wall and internal abdominal structures, particularly the lymphatics. The fungus failed to grow on bouillon or glycerin agar. The descriptions and illustrations of this case show characteristics making it probable that the disease was actinomycosis rather than mycetoma.

Vincent (1894) reported a case of ochroid variety and cultivated a *Streptothrix* from the lesions. The granules resembled *Actinomyces,* being yellowish-white in color and insoluble in potash and in acetic acid; when stained by Loeffler's method or by fuchsin and enlarged 400 to 500 times, they were seen to be made up of filaments and mycelial débris. The peripheries of the granules showed filaments of radiating types. Vincent considered the parasite to be a *Streptothrix* and named it *S. maduræ.* The filaments when examined under the high powers of the microscope were shown to contain elements of varying size, some being so small as to resemble micrococci. Scheube's abstract is so satisfactory that we have quoted it, after comparing the original.

The material used by Vincent for the preparation of cultures was taken by him direct from the diseased tissue. "It exhibited but little growth in bouillon, but infusions of hay and straw (15 grams to 1 liter) not neutralized, and therefore with an acid reaction were found to be suitable nutritive media. The same holds good for meat soups, to which yellow turnips, carrots, and more especially potatoes (20 grams to 1 liter) have been added, after previous filtration and sterilization. The temperature should be about 37° C; if it is over 40°, growth stops. The cultures, which are placed in wide tubes or Ehrlenmeyer's flasks, to which air is accessible, exhibit small, gray flakes, round or flat, on the fourth or fifth day; these cling to the wall and bottom of the tube, and after from twenty to thirty days attain the dimensions of a small pea. Some of the flakes exhibit a brown tinge in the center, while others that lie near the surface of the liquid become pink or red after a month or two. The nutritive fluid never becomes cloudy, for most of the flakes lie at the bottom, where they form a covering which exceeds 0.5 to 1 centimeter in thickness. The nutritive fluid, previously acid, in time assumes an alkaline reaction, and becomes pale-blue.

"In ordinary gelatin the *Streptothrix maduræ* only exhibits a weak growth of white colonies along the inoculative puncture on the surface. Vincent considers that the best solid nutritive soil is 100 cubic centimeters of an infusion of hay or potatoes, with the addition of 9 grams of gelatin, 4 grams of glycerin, and 4 grams of glucose, neutralized and sterilized in the usual manner. The *Streptothrix* does not liquefy the gelatin. On this soil fine, luxuriant, round, glazed colonies of a white or pale yellow color are obtained, which later on often assume a pink or even red color. If very many colonies form on the gelatin they remain small, but if fewer are present they attain almost the size of a pea. They then resemble an inoculation pustule sunk in the middle, and are of a white color, the margins assuming a red tint. Old cultures fade and become dull white. The colonies are of a horny consistency, and cling very firmly to the nutritive media.

"The parasite grows fairly well on milk without coagulating it, though it becomes slowly peptonized. On eggs and serum, however, it does not flourish at all.

"On potatoes, from the fifth day, and at 37°, small, uncolored, or whitish prominences are seen, which assume a pale, reddish color after a month. This gradually deepens and becomes sometimes a bright pink, sometimes orange-color or red, sometimes a fine, dark red. This occurs particularly when the potatoes have a strong acid reaction, whereas on some potatoes there is no coloring at all. On carrot media the red coloring is more pronounced than on potato.

"Some colonies are covered by a fine, white dust which consists of spores.

"The parasite is aërobic. The parasite stains well with basic aniline stains, more faintly with safranin and eosin. It can also be stained according to the methods of Gram and Weigert. A solution of iodine stains it yellowish-brown, while hæmatoxylin causes it to assume a violet color.

"The fungoid filaments are finer in the cultures than in the little bodies described above; their breadth does not exceed 1 μ. The irregular swellings and contractions observed in the little bodies are not observed in the cultures, which, however, when two weeks old often exhibit a series of spores at the end of the filaments. They are ovoid, light refracting and about 1.5 μ in breadth and 2 μ in length. They stain well with aniline dyes and according to Gram's method. They exhibit very little resistence to heat; they are killed in three minutes at 85°, and in five minutes at 75°. The cultures that do not carry spores die in three to five minutes at 60°.

"The formation of spores takes place best where the mycelium comes into contact with the air, and this is the case in fluid mutritive media as well as on potato. Cultures with hay broth are the most suitable for this purpose.

"One may actually see the spores develop in fresh broth and in suspended drops.

"The cultures are very resistant to desiccation; even after a period of twenty-one months they exhibited a capacity for development.

"The *Streptothrix maduræ* does not prove pathogenic to animals (rabbits, guinea pigs, mice, cats). Bocarro's experimental transmissions with fresh material of both varieties were negative in rabbits as well as in dogs. Nocard also inoculated with cultures guinea pigs, rabbits, pigeons, hens, dogs and sheep, by intravenous, subcutaneous and intraperitoneal inoculations, with negative results in every case.

"By means of parallel cultures arranged and kept under exactly the same conditions, Vincent confirmed the difference between the *Streptothrix maduræ* and the actinomyces, as shown on the table.

"Boyce and Surveyor likewise succeeded in cultivating a streptothrix in a case of the white variety; it exhibited a condition similar to that cultivated by Vincent, but no ramifications and spores could be discovered.

"Legrain could not cultivate colonies in infusions of hay, but succeeded in doing so on gelatin with malt and peptone. The colonies were round, prominent, glazed, very adherent to the lower surface, at first pale, then golden-yellow, and soon after became of a vivid red color and finally became decolorized.

"In the nodules which exhibited suppurative degeneration, Vincent found *Staphylococcus pyogenes albus* and *aureus* in addition to the parasites described."

Vincent gives the following comparative table of the cultural characteristics of *S. maduræ* and *Actinomyces:*

TABLE No. I.—*Comparison between S. maduræ and Actinomyces.*

No.	Cultures or inoculations.	Streptothrix maduræ.	Actinomyces.
1	Peptonized beef tea	Moderate growth	Luxuriant growth.
2	Sterilized infusion of hay or straw.	Principal nutritive soil; growth rapid (4 days) and luxuriant.	No growth.
3	Ordinary peptone-gelatin	Does not liquefy	Liquefies.
4	Gelatin with infusion of hay.	Very quick growth; the culture becomes pink or red on the surface.	Whitish, very weak culture.
5	Glycerin gelatin	Colonies which at first are white, then pink or red, and umbilicated.	At first white, later on grayish spots; folded.
6	Potato	Bright pink, vivid or dark red culture; does not brown the substratum.	Close, wart-like yellow and white, black-edged colonies; potato becomes brown.
7	Cabbage, yellow turnip, carrot.	Growth	No growth.
8	Serum	No growth	Growth.
9	Egg	do	Do.
10	Cultures in areas deprived of air.	do	Facultatively anaërobic.
11	Inoculations	Not transferable to any animal	Transferable to rabbits, guinea pigs, calves.

S. maduræ according to Vincent differs culturally from the fungi isolated by Nocard,[15] Almquist,[16] and Eppinger.[17] The Nocard organism develops rapidly in peptone-bouillon and it does not grow in hay infusion. The cultural characteristics upon agar and potato present no analogy. Nocard's organism is inoculable in guinea pigs.

Vincent also discusses the question of the identity of actinomycosis and mycetoma and notes the following differences: Potassium iodide does not give the same positive results with Madura foot as it does with actinomycosis. Different results are obtained with the two parasites, not only morphologically but also in cultures in various media and in inoculation experiments.

[15] *Ann. d. l' inst. Pasteur* (1888), 2, 293.

[16] *Ztschr. f. Hyg. u. Infectionskrankh.*, Leipz. (1890), 7, 189.

[17] *Wien. Klin. Wchnsch.* (1890).

Vincent and Gémy (1896) mention a second case of ochroid variety and the cultivation of the same *Streptothrix* already reported by Vincent. Preliminary mention was also made by these authors at the Congrés de Dermatologie et de Syphilis in Paris on April 25, 1892.[18]

Shattuck (1898) reported to the Pathological Society of London a case which he designated as *Mycetoma papillomatosum.* The skin exhibited a pronounced papillomatous condition, and secondary papillæ with sinuses and fungi were found within the larger growths. Maitland (1898) reported a case involving the thigh and abdominal wall of a man, and Smyth (1898) published one involving the tissues of the neck.

Plehn, according to Scheube, observed three cases in Suahelinese on the Tanga coast; however, probably these had been carried to that point from India. Vincent mentions the fact that Layet encountered the disease in Chile and Crookshank (1897) discussed the infection after collecting data obtained by the examination of a specimen sent from India. Boccaro (1893) analyzed one hundred cases, and in seventeen instances he saw evidence of a prick by an acacia thorn. In many of these cases the thorn itself was found. The same author also reports a case of mycetoma involving the tissues between the vertebral column and the scapula. Descriptions of the infection are also given by Delbanco (1897 and 1898); by Huntly (1890 and 1899), Kobner (1891), Paltauf (1894), Ruelle (1893), Shah (1893) and the pathology is fully outlined by Unna (1897).

The first case in America was reported by Kemper and Jameston,[19] but the article is not available to us.

Adami and Kirkpatrick[20] studied a case of the ochroid variety in Canada and considered the granules to be identical in appearance with those from actinomycosis, and Hyde, Senn and Bishop (1896) reported one from Chicago. In the latter instance the fungus suggested *Actinomyces.* No club-shaped forms were seen, and in sections the central portion of the granules stained faintly with hæmatoxylin. Under high power, delicate, radiating filamentary threads were observed in the masses. Delbanco, (1897) (according to Scheube) who studied materials sent him by the authors reporting this case, concluded that it was actinomycosis and not Madura foot. The record of Pope and Lamb's case (1896) is not available to us. Arwine and Lamb[21] (1899) mention another case in a native of Texas. Dr. Carroll who examined sections from the specimen considered the disease to be actinomycosis.

J. H. Wright[22] (1898) reviewed some of the literature of the subject and reported one case of the melanoid variety from the service of Dr. Beach in the Massachusetts' General Hospital in Boston. Wright's patient was an Italian woman who had resided in the United States for several years, but the disease had been noticed less than a year before she came under his care. The clinical manifestations of the infection and the pathological alterations in the specimen taken from this patient, as they are described by Wright, are very similar to those which have been given by other authors. The description of the granules is very clear and Wright states that when bleached and softened by solutions of sodium hypochlorite or of potassium hydroxide, they are "found to consist of a mass of fungus structures, together with more or less brown, pigmented imbedding or investing material, as described and figured by Boyce and Surveyor." No spores were observed and the test for hæmin in the granules was negative. The latter were deeply stained by the basic aniline dyes, but not by hæmatoxylin. "The Gram stain and the Weigert fibrin stain, or modifications of them, stain the substance of the granules to a variable extent depending on the amount of the decolorizing agents applied." Wright cultivated a "*Hyphomycete*" from approximately twenty-five of sixty-five of the black granules. *Stapylococcus albus* also grew in most of his cultures. The growth developed from the granule used in inoculation and appeared after an interval of four or five days or even later. Growth occurred as follows in most of the media used:

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[18] *Ann. de dermat.* (1892), May.

[19] *Am. Practitioner* (1876), 577.

[20] *Trans. Ass. Am. Physicians* (1895), **10,** 92, and *Montreal Med. Journ.* (1905–6), **24,** 485.

[21] *Am. J. Med. Sc.* (1899), N. S. **188,** 393.

[22] *J. Exp. Med.* (1898), **3,** 421.

Potato.—A dense, widely spreading, coherent layer of velvety surface; pale brown in the central portion and white at the edges. Small droplets of dark, coffee-colored fluid develop on the surface of the cultures. The medium becomes dark brown and very moist.

Bouillon.—Growth proceeds from the inoculated material in fine, radiating filaments and produces a puffball-like appearance, and eventually the whole fluid is filled up with radiating mycelia, the fluid becomes a deep coffee-brown color and a mycelium layer develops on the surface.

Potato infusion (20 grams boiled in water, with a finished filtered product of 1,000 cubic centimeters and not neutralized).—Growth is much the same as in bouillon, but no surface layer appears and in old cultures there are found numerous, black granules about 1 millimeter or less in diameter in the midst of the mycelium.

"These granules consist of closely packed, spherical or polyhedral cells, together with some short, thick, segmented hyphæ. The walls of these cells have a black appearance and masses of them are black and opaque under the microscope. Wright considered these granules to be "masses of interlacing hyphæ whose segments have been much shortened, and widened and otherwise changed." They were examined by W. G. Farlow and pronounced to be 'Sclerotia.'

Agar (plain and glucose).—"Growth appears as a mesh work of widely spreading filaments, of grayish color, on the surface of the medium." "Sclerotia" develop in old cultures, and in slant cultures growth only takes place on the surface.

Morphologically the *Hyphomycete* consists of long, branching hyphæ from 3 to 8 μ in diameter. Young forms show delicate transverse septa and older ones swellings at the points of branching and the hyphæ may appear as a string of oval-ended, plump segments. The filaments have a definite wall and branching occurs by lateral budding. No spore-bearing organs were observed.

Animal inoculations.—"No results were obtained from the inoculation of animals with the original granules or with cultures."

Histology.—"The tissues composing the nodules consist essentially of a formation of more or less atypical connective tissue in various stages of development in which foci of suppuration are present in association with granules.

"Some of the granules lie in small cavities containing polynuclear leucocytes, loose epithelial cells and cellular detritus. These cavities may be lined either by a wall of vascular granulation tissue or by massess of epithelioid cells, together with multinucleated giant cells. Other granules are closely invested by a zone of epithelioid and giant cells, and outside of this there may be an infiltration with lymphoid and plasma cells. The nodule thus formed about the granule resembles very closely a tubercle in structure. The giant cells are often of large size and may have a peripheral arrangement of their nuclei. They are a very prominent element in the lesions.

"The primary effect produced by the parasites upon the tissues seems to be the development of nodules of epithelioid cells and of giant cells from the tissues immediately about them. Later, suppurative processes occur in the nodules and abscesses are formed, which in the tissue give rise to the development of granulation and connective tissue in large amount."

Unna and Delbanco's (1900) discussion of the subject is mentioned by MacLeod. Brumpt (1901) reported a case of the black variety of mycetoma from Suakim, Sudan. He gives the usual description of the granules. The grains are composed of an enveloping membrane made up of mycelial filaments. The inner portion is very friable and easily crushed. Cultures in hay infusion and bouillon gave negative results. Fourlerton and Jones (1902) published an exhaustive article dealing with the pathogenesis of the *Streptothrix* group in general; the authors in discussing *Mycetoma* consider *Streptothrix maduræ* Vincent as the cause of the ochroid variety, and J. H. Wright's *Streptothrix* as that of the black type.

Laveran[23] studied an anatomical specimen of the black variety sent to him by Bouffard. He found and described a *Streptothrix* in the granules and named this organ *S. mycetomi*. The author does not report any experiments in the cultivation of the fungus.

Madden[24] published two cases of the pink variety of mycetoma occurring in the Sudan. In one of the patients the primary lesion was in the thigh, and later the disease involved the abdominal wall. Pinoy succeeded in one month in cultivating a *Streptothrix* in anaërobic, sweetened bouillon. With these cultures he was able to infect a pigeon's foot.

Scheube (1903) discusses the question of the identity of actinomycosis and Madura foot as follows:

"I need only recall the different size and coloring of the fungoid masses, in the two diseases, their different localization, and the difference of their course, which in Madura foot is more benign and more chronic than in actinomycosis. The pronounced tendency also for actinomycosis to spread to other near or distant parts of the body, to attack the internal organs, and the transmissibility of actinomycosis to healthy persons, are qualities which, at least according to our present knowledge, are not possessed by Madura foot. Moreover, the *hyphomycetes* of Madura foot are more delicate, and stain remarkable well with hæmatoxylin, showing the prisms and columns described above as staining with difficulty. Actinomycosis, on the other hand, is not stainable with hæmatoxylin and develops clubs and knobs which are difficult to stain."

Manson (1906) in discussing *S. maduræ* Vincent considers that "though closely allied to the better known fungus (*Actinomyces*), apparently it is not specifically identical with it."

Nicolle and Brunswic-LeBihan[25] report mycetoma in an Arabian woman. The original lesion was produced by a barley spore introduced one year before the mycetoma was established, and Nicolle and Pinoy[26] report a case of the ochroid variety of the disease in an Arab. They described and cultivated a *Streptothrix* from the lesions. The organism was grown upon sweetened hay infusion and developed on the surface of the medium as a light gray superstratum upon a brownish-red membrane. The authors consider carrots to be the best medium for rapid development of the organism, and growth occurs best at 37°, appearing as a white scum which rapidly becomes yellowish. Small spots of various colors also develop, according to the acidity of the media, the colors varying from grayish to brick red. Roux tubes in which were placed reeds and water, sterilized, constitute a favorable culture medium. In less than forty-eight hours a glaucescent growth covers the surfaces of the reeds.

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[23] *Bul. Acad. de Med.* (1902), (3), 47, 773.

[24] *J. Trop. Med.* (1902), 5, 243.

[25] *Bul. Acad. de Med.* (1906), (3), 55, 132.

[26] *Arch. d. Parasit.* (1906), 10, 437.

Potato.—A white growth develops in forty-eight hours, this increases up to eight days, becoming brownish. The medium becomes dark.

Gelatin.—On this medium the growth is very feeble and no liquefaction occurs.

Agar and Sabourand's gelose.—The growth consists of a brownish, folded membrane frosted with white, slowly becoming yellow.

Animal experiments.—Subcutaneous inoculation of monkeys and rabbits gave negative results. Subcutaneous inoculation in the foot of a rat produced small granulations which contained mycelial filaments; these granulations were gradually reabsorbed. Intravenous inoculations in rabbits were without result. The author discusses the question of the etiology of the disease and the identification of the fungi.

E. Brumpt[27] has written a critical review of the entire subject with particular reference to the parasites. He divides the fungi into two genera and several species, gives the generic and specific diagnosis of each, and places the previously described parasites in what he considers their proper position. He considers mycetoma to be a clinical type of disease which may be caused by any one of the several species of *Streptothrix* which have been described by various authors. The work is very exhaustive and the plates are excellent, but after close study it seems to us that his classification has been made upon insufficient data.

Foulerton (1907) in his recent and complete study of the pathology of *Streptothrix* infections in general, divides the group into several classes according to the lesions produced, the cultural characteristics, and so on; in one of these groups he includes *S. maduræ* Vincent and the other allied organisms which from time to time have been published as the etiologic agents in Madura foot. The author was unable to demonstrate acid-fast properties in cultures of *S. maduræ* with which he worked. In considering the acid-fast properties of *Streptothrix* in general, the author states that the number of these organisms is still too small to justify fully definite conclusions, and in addition to *B. tuberculosis* and other well known bacilli of the same type, he mentions *S. eppingeri, S. nocordii, S. capræ* as well as Sabrazis' and Rivère's organisms as having a similar acid-fast character. As our own *Streptothrix* is of this class of acid-fast organisms, a comparative study of the group is interesting and will be taken up more in detail in a subsequent paper.

Wright[28] in Osler's Modern Medicine discusses the question of the existence of two varieties of the disease. This author states that "at the present time the ochroid or pale form of mycetoma must be regarded as actinomycosis of the part." However, in the melanoid variety he recognizes the specific fungoid nature of the affection, although, as he himself states, the etiology of the disease remains to be proved by animal experimentation. Wright[29] was probably the first one to cultivate the fungus of the black variety. Its cultural characteristics have been given and are shown in the parallel column of Table II facing page 500.

[27] *Arch. d. parisit* (1906), **10**, 489.

[28] Osler's Modern Medicine (1907), **1**, 344.

[29] Loc. cit.

A very recent article by MacLeod[30] discusses the etiology of the disease in the following manner: "It is caused by the presence in the skin and underlying tissues of a *Streptothrix* closely allied to, but differing from, actinomycosis. The name *Streptothrix maduræ* has been given it by Vincent. This fungus forms the 'fish roe' like granules of the white variety, which is the common form of the disease." The author in describing the nature of the black variety states that there is a considerable variance of opinion, but he does not adhere to the belief that its fungus is a degenerative condition of the infection produced by the white type, but rather that it is a different species, as is claimed by several writers. MacLeod also recognized a red variety of the mycetoma in addition to the ochroid and melanoid types. In considering the differential diagnosis of the disease from actinomycosis the author states: "Actinomycosis is a disease which is transmitted from animals to man, and can be inoculated in animals; it occurs in temperate latitudes, often runs a rapid course, affects internal organs and mucous membranes, and has yellow granules in its discharges; Madura foot is confined to man, has not been successfully inoculated in lower animals, occurs in the tropics, runs a slow course, does not become generalized, and presents granules of various colors."

Caminiti[31] (1907) reports a new *Streptothrix* and discusses very fully the whole group of *Streptothrix;* however, but little attention is given to mycetoma.

DISCUSSIONS AND CONCLUSIONS.

The study of our case of Madura foot, a report of which is given in the first part of this paper, after a careful review of the literature, establishes for the first time definitely so far as we have been able to determine, the etiology of mycetoma according to the usual requirements of investigators, including the transmission of the disease by animal experiment. The causative organism we have determined to be a *Streptothrix,* and as it apparently differs from previously described fungi of the same genus we have named it *Streptothrix freeri.* We believe this investigation fully establishes the etiologic importance of *S. freeri* in one case of the pale or ochroid variety of Madura foot, but nevertheless we are of the opinion that it does not finally settle the entire question of the etiology of the disease. Some of the most important questions open for discussion are as follows:

1. Is *S. freeri* sufficiently different from previously described organisms to entitle it to be classified as a new species?
2. Is mycetoma a distinct disease etiologically, or is it one type of manifestation of more than one species of *Streptothrix* infection?
3. Are the "ochroid" and "melanoid" varieties different stages of a single etiologic entity, or are they due to different species of *Streptothrix*?
4. What is the relation of mycetoma to actinomycosis?

In spite of all of the admirable older investigations on the subject and giving all due credit to the writers of former contributions, there are but two articles dealing with the etiology of the disease which are sufficiently clear and which follow required methods closely enough to be of value in a comparative study. These are Vincent's report on *S. maduræ* as the cause of the infection in the ochroid variety, and Wright's article reporting a *Hyphomycete* as the etiologic factor in the melanoid type of the disease. The work of both these authors seems to have been carefully carried out and their conclusions appear to be sound, but in neither investigation did the authors establish the etiologic relation of their organisms to Madura foot by experiments on animals. However, leaving this omission out of consideration and studying the cultural characteristics of their fungi alone, the evidence seems to be conclusive that *S. freeri* differs from both of their organisms. This is not only so, but when we compare its characteristics with the description of other species of pathogenic *Streptothrix* which have been cultivated by others, we find our organism clearly to be distinct. The table opposite gives a comparative summary of the characteristics of six of these *Streptothrices*, and it includes those of *S. actinomyces*.

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[30] Albutts' System of Medicine (1907), 2, 754.

[31] *Centrbl. f. Bakteriol.* Orig. (1907), 44, 193.

There can be no reasonable doubt but that all types of mycetoma are due to *Streptothrix* infections, but whether all the forms are caused by an infection with a uniform organism or whether more than one species plays a part in the disease, can not now positively be stated. However, it is very probable that Madura foot may be produced by any one of several species of *Steptothrix*, and that lesions of etiology identical with the ones occurring in the foot may be produced in various parts of the body. Such infections are now occasionally recognized. It seems likely that some of these lesions, located in places other than the foot, are not considered to be mycetoma, more because of their location than because of any specific differences in the parasites. However, if the methods employed by observers who have reported mycetoma in parts of the body other than the foot are examined, it is certain that the diagnoses of these infections were based upon morphologic considerations of the *Streptothrix* as a whole, and therefore were not conclusive as to the determination of *species.*

S. freeri is as pathogenic for monkeys, when it is inoculated in the deep tissues in other parts of the body, as it is when injected into the foot, where it produces typical mycetoma clinically and pathologically. It seems probable that mycetoma most often occurs in the foot because of the accidents to which that member is exposed and in this connection it may be stated that the right foot is more frequently affected than the left one. If the general infections produced in animals by *S. freeri* prove to be experimentally practical with other types of the disease, then Madura foot becomes a variety of streptothricosis differing from other types more in anatomical position than in specific etiologic distinctions.

The review of the literature which has been given shows that there is much difference of opinion in regard to the etiology of Madura foot.

Oversized Foldout

The only important data tending to show that different types of the disease exist are found in the varying color of the granules from the different varieties and the results of Wright's cultivation experiments. While Wright could not establish the etiologic importance of his organism, it is probable that it was the cause of the disease in his case; this being true, the etiologic identity of the two varieties must be questioned until further experimental work is done with both Wright's and our own organisms.

One of our experiments on monkeys is suggestive. A fair number of small, black granules were produced in the tissues of monkey number 3267 inoculated subcutaneously, and a less decided, but positive, variation in the color of the granules has been noticed in other inoculated animals.

The statement which has often been repeated, that the ochroid variety of mycetoma is actinomycosis, is not supported by the weight of evidence in the literature, and it is positively disproved by our work.

There is no doubt but that actinomycosis has been mistaken for Madura foot, both of the ochroid and black variety, in some of the reported cases, but it is equally certain that *Actinomyces hominis* is a different species of *Streptothrix* from those producing mycetoma. Furthermore, the two diseases show decided differences in clinical manifestations and in the pathologic findings.

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ILLUSTRATIONS.

PLATE I.

FIG. 1. Madura foot, mycetoma.
2. Monkey's foot after ten days. Inoculated with cultures from the lesions of foot shown in Plate I, fig. 1.
3. Monkey's foot after fifteen days. Inoculated with scrapings from the lesions of foot shown in Plate I, fig. 1.

PLATE II.

FIG. 1. Section from the foot of the original case; stained preparation. 1 × 75.
2. Section from the foot of the original case; stained preparation. 1 × 100.
3. Section from abdominal tumor of dog number 3390; stained with Sterling's gentian-violet iodine solution (1–2–200) decolorized with aniline oil. 1 × 390.

PLATE III.

FIG. 1. Fresh smear from the lesions of the original case. 1 × 850.
2. Fresh smear from a thirty days' culture on glycerin-agar. 1 × 850.

PLATE IV.

FIG. 1. Drawing of a ten days' culture of *Streptothrix freeri* on glycerin-agar.
2. Potato culture of *Streptothrix freeri* showing ten days' growth.

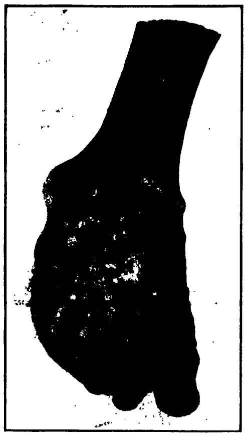


FIG. 1.

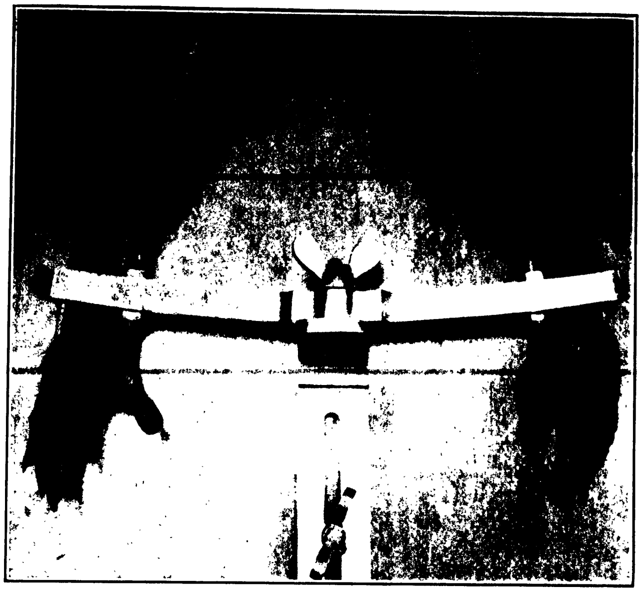


FIG. 2.

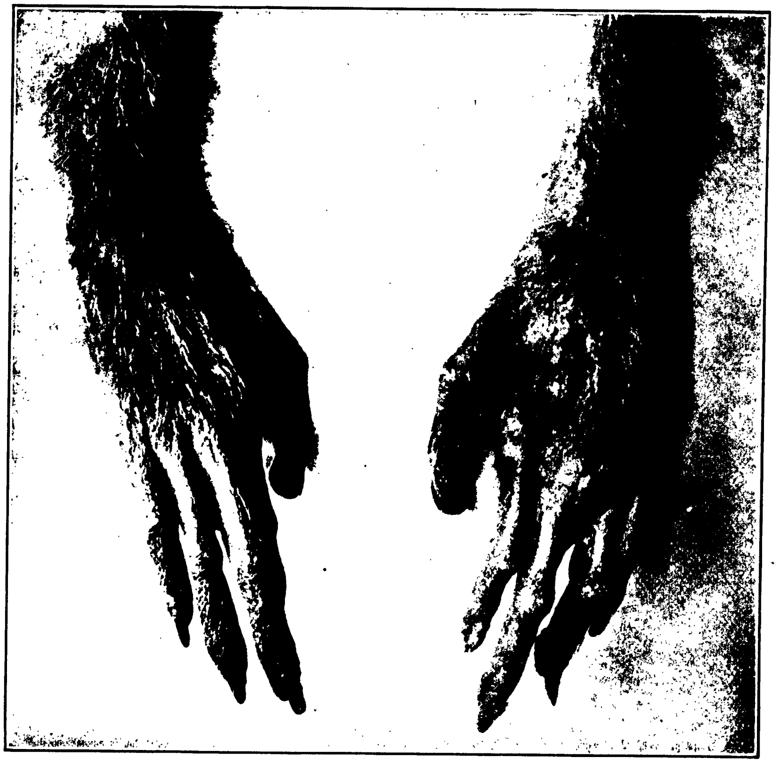
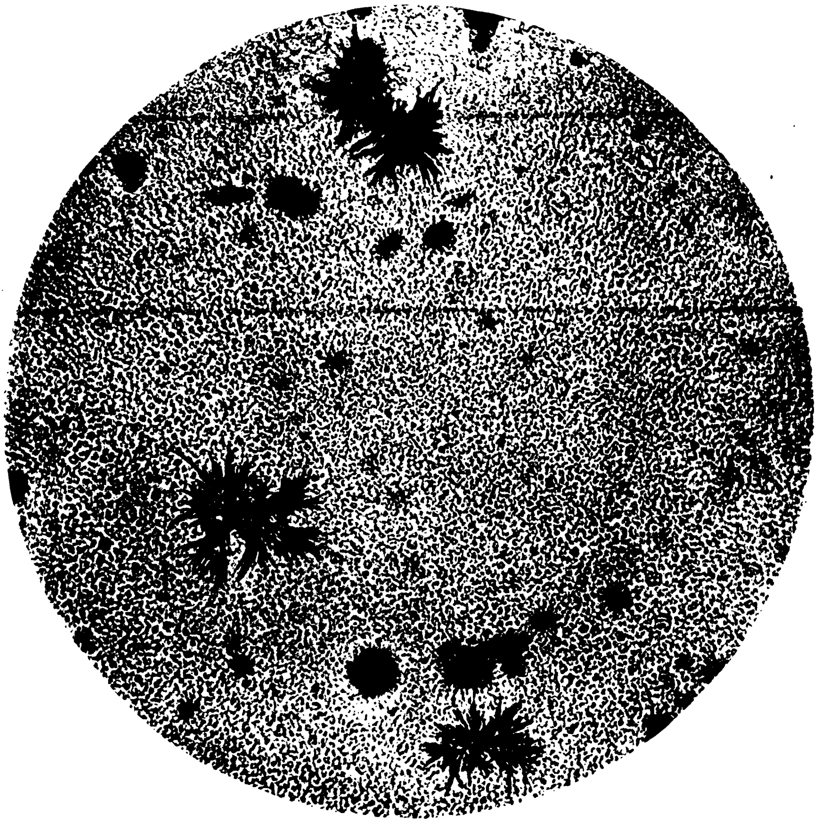


FIG. 3.

PLATE I.



Fig' 1.

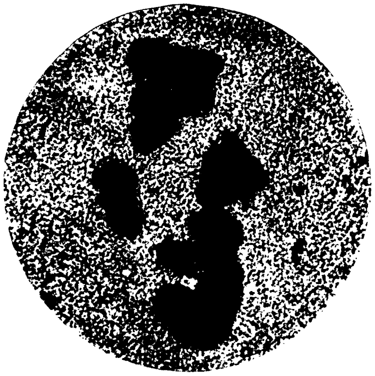


Fig. 2.

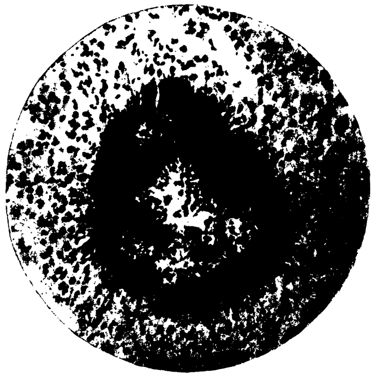


Fig. 3.

PLATE II.

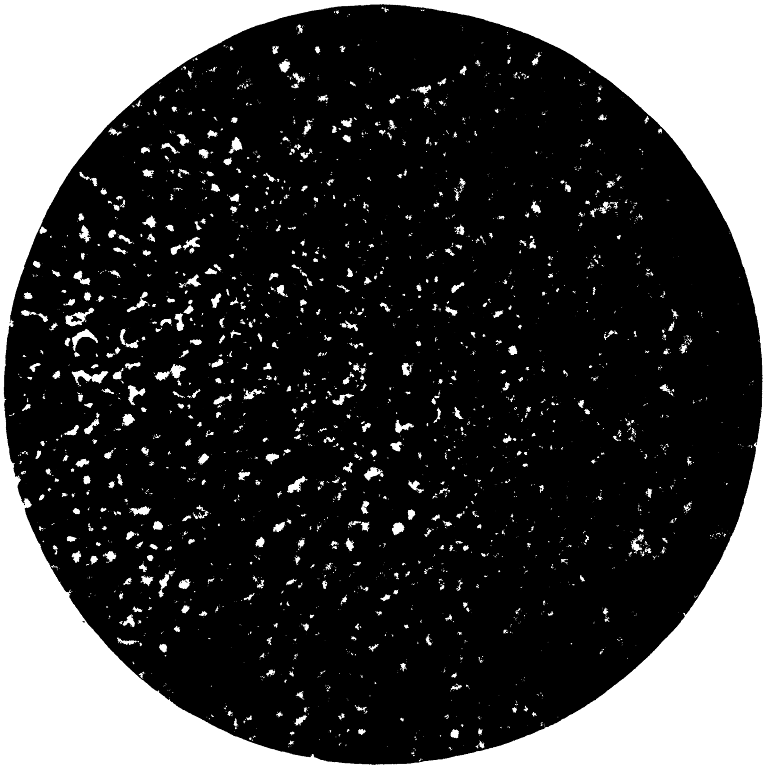


FIG. 1.

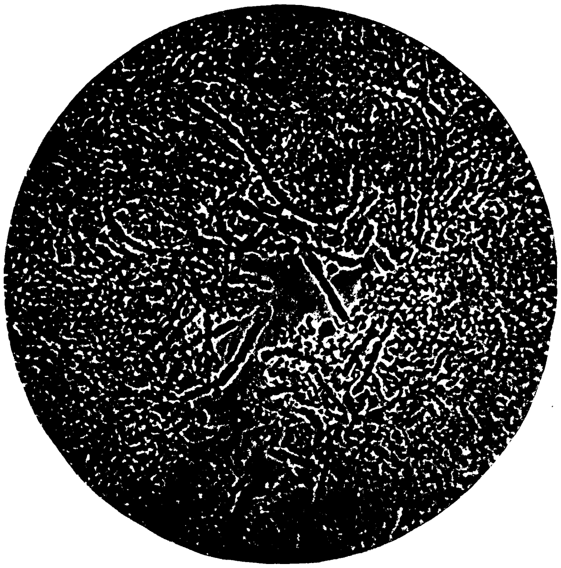


FIG. 2.

PLATE III.

FIG. 1.

FIG. 2.

PLATE IV.

EXPERIMENTS IN MALARIAL TRANSMISSION BY MEANS OF MYZOMYIA LUDLOWII THEOB.

By Charles S. Banks.

(From the Entomological section, Biological Laboratory, Bureau of Science, Manila, P. I.)

1. Introduction.
2. Breeding Places of the Mosquitoes.
3. Life History of Myzomyia ludlowii Theob.
4. Mosquitoes and Malarial Parasites.

INTRODUCTION.

Physicians in the Philippine Islands have for several years been desirous of determining with greater certainty the exact species of *Anophelinæ* responsible for the transmission of malaria in this region, although as far as I am aware no definite experiments looking to this end have ever been carried on.

In the year 1906 a very severe epidemic of æstivo-autummal fever, resulting in several deaths both among the American and Filipino workmen, broke out in a camp some 6 kilometers from Manila on the line of the new waterworks excavations. I went to the scene of the trouble to study the features of the infection, but not until some time after it had been decided to abandon this camp and all the workmen, including also the families of the Filipinos, had moved away. A very brief visit of two or three hours, made at a time previous to the breaking up of the camp, enabled me to determine the actual state of existence of these people and to find out that practically none of the Filipinos and very few of the Americans used mosquito nets. A great many specimens of *Nyssorhynchus barbirostris* Theob., were found among clothing in the huts of the workmen.

A reconnaissance of the immediate vicinity of the camp at the time of the second visit revealed the abundant breeding places of the species of mosquito just referred to, but no further attempts were made definitely to fix the blame of malarial transmission upon the species found.

In December, 1906, word came from the United States naval station at Olongapo, Zambales, that malaria was very prevalent among the large body of marines in barracks at that place and that it was of a very pernicious type. It had been noted for some time previously that companies going for target practice to the marine rifle range at Maquinaya, some 5 kilometers north of Olongapo on the east shore of Subig Bay, invariably returned to Olongapo with a large percentage of their personnel infected with malaria, in one instance as many as 85 per cent having the fever.

It was not possible, owing to pressure of other work in Manila, for me to reach Olongapo before the 1st of April, so that upon my arrival I found the disease to have diminished markedly, although cases were by no means of rare occurrence.

GEOGRAPHY AND TOPOGRAPHY.

The naval station of Olongapo is situated at the southeast end of the small island of that name, lying in the eastern part of Subig Bay and separated from the mainland of Luzon by the Kalaklan River. It is really one of a series of deltas formed at the mouths of the Kalaklan, Santa Rita and Biniktigan Rivers. The region roundabout is hilly, often to the coast, the main axes of the hills running at an angle with the coast line and having broad valleys between them, especially on the east shore of the bay. More or less sluggish streams flow through these valleys and upon reaching the coast they naturally spread out, forming areas of mangrove swamp in which a certain species of large crustacean has built up mounds of earth which in time, owing to vegetation and the further deposit of earth by the rivers, have united to form a more or less level tract having an elevation of from 3 to 10 decimeters above mean tide. Numerous channels are cut through these tracts of land at the time of floods, and when the tides are high the waters of these channels are continuously mixed with that from the sea. At no time is the water in or around these areas fresh, and where the tide recedes it leaves the landlocked pools to become more salty by evaporation. The shores for several kilometers around Olongapo in all directions are of this character, and this condition is only relieved by the terminal talus slopes of the surrounding hills.

Engineering operations carried on by the Navy with reference to harbor improvements at the station, have altered the course of the Biniktigan River while causing large bodies of water to become more landlocked than previously, with the result that now the area of stagnant or semi-stagnant water in the vicinity of the station is about doubled.

The same condition obtains at the Maquinaya rifle range, except that the swamps at this place lie farther landward, whereas the camp itself is located on a sandy beach, upon which fair sized trees of various species grow.

LOCATION AND BUILDINGS.

The navy-yard proper is located on a sand spit and is surrounded by a wall built in Spanish times; all the offices and the quarters of most of the naval and marine officers and the hospital are located within this wall. Outside, and along the west beach of the island are the other marine officers' quarters and eastward from these, at a distance of some 120 meters, are the five large buildings erected for barracks for the troops. Four of these barracks are within a few meters of the swamp regions. They are built on cement piers about 1 meter from the ground level and are well ventilated. Sewage is carried into Subig Bay by pipe lines and there is no stagnant water in the immediate vicinity. Each building is provided with a tank on the upper veranda into which water is pumped for bathing purposes and for flushing the closets. The ground area beneath and around the barracks is kept in a scrupulously clean condition, many of the posts and piers being frequently whitewashed and no weeds are allowed to grow. The parade ground at the west and south of the barracks is not yet finished and therefore it is cut in many places by trenches and ditches into which tide water finds its way and where *Culex microannulatus* Theob., and *Myzomyia ludlowii* Theob., breed in great numbers. Larvæ of both the above-mentioned species of mosquitoes were found at the Maquinaya rifle range, freely breeding in salt water less than 60 meters from the camp, which consisted of tents for both officers and men.

According to regulations, all men are obliged to sleep under mosquito nettings, but I am of the opinion that these do not completely serve their purpose in preventing the men from being bitten, for few sleep so quietly that at some time during the night their arms or legs do not come in contact with the sides of the narrow nets, thus offering a ready mark for mosquitoes.

PLAN OF WORK AT OLONGAPO.

It was decided that the first requisite was the discovery of all species of mosquitoes which might play an important rôle in the transmission of disease, as well as to study the conditions under which the insects breed most readily. A considerable amount of time was consequently spent in reconnaissance in the neighborhood both of Olongapo itself and the rifle range at Maquinaya. During this period mosquito larvæ were collected and bred, and breeding grounds mapped out. This work enabled me to narrow the species of *Anophelinæ* down to *Myzomyia ludlowii* Theob., as the most probable transmitter of malaria.

FACILITIES.

The breeding grounds of *Anophelinæ* were so near to the laboratory where my indoor investigations were carried on, that it was an easy matter to go from the outdoor cages to the workroom several times a day if necessary. At first, certain places were simply watched daily and the development of the larvæ and pupæ noted, but as work went on it was found desirable to isolate individuals and eggs in order to determine with exactness the various periods of growth. I took with me from Manila most of the portable apparatus necessary, but many features which needed to be constructed on the spot were furnished by the naval authorities, all of whom, without exception, did everything in their power to aid my work. I wish in this place to express my thanks for their assistance.

SUBSIDIARY INVESTIGATIONS.

On the 22d of April a battalion of marines, fully equipped for heavy marching, left Olongapo under the command of Maj. E. K. Cole, United States Marine Corps, for a military reconnaissance of the region around Mount Pinatubo to the north of Olongapo and distant some 48 kilometers. This expedition from the time of starting until the return to Olongapo occupied five and a half days, during which time the whole command slept at no one place for two consecutive nights. We left the seacoast at the town of Subig 12½ kilometers north of Olongapo at the head of Subig Bay and struck inland, there being a continuous and gradual rise in the land until the second day, when the country became strictly mountainous. The night camps were always pitched in the vicinity of running water, but in no case was stagnant or semistagnant water encountered during the trip. A few mosquito larvæ were found at Santa Fé in the river near the camp, but the specimens collected died before reaching maturity. However, they were not Anophelines.

On the last night of the expedition (April 26) at a small place south of Castillejos, Zambales, the sleepers were annoyed by considerable numbers of *Devoidya joloensis* Ludlow (*D. fusca* Theobald, var. *joloensis* Ludlow).[1] This species probably breeds in the rather sluggish river near this place, although a search did not reveal the larvæ at that time.

These mosquitoes were the only ones encountered on the trip and it is noteworthy that no single individual of the entire expedition, comprising some one hundred and twenty-five men, was attacked by malaria subsequent to his return to Olongapo, within the usual incubation period of the disease; in fact, a careful observation of the men of the command for two months after their return showed that, where there was any malaria, it was a recrudescence of the æstivo-autummal type and in individuals who had been suffering from the disease for some time before this trip.

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[1] Theobald says in *Mono. Culic.* (1907), 4, 165, that this variety merits specific rank and I have so indicated it.

THE BREEDING OF MOSQUITOES.

GENERAL CONSIDERATIONS.

Most of the species of mosquitoes breed in fresh water; in ponds, sluggish streams, the margins of lakes and swamps or in cisterns, wells, water tanks and all other receptacles for collecting water around houses, which are not covered by wire gauze or other mosquito-proof covering. Certain species breed only in open meadow or marsh waters, others only in woodland waters and again others only in receptacles in or near houses. Examples, respectively, of these three classes in the Philippines are *Nyssorhynchus barbirostris* Van der Wulp, *Desvoidya fusca* Theob., *Worcesteria grata* Banks, *Culex fatigans* Wied., and *Stegomyia persistans* Banks. The total number of known species of Culicidæ found to breed in salt or even brackish water is so very small that the finding of a true anopheline breeding under such conditions is remarkable.

Only the following species of *Culex* are known to multiply in salt or brackish waters in the United States: *C. solicitans* Walk., *C. salinarius* Coq., *C. tæniorhynchus* Wied., and *C. cantator* Coq. In Europe Bancroft and Ficalli record larvæ of *Culex salinus* Fic., from salt water, while Theobald adds *Culex marinus* Theob.

Three species of mosquitoes have been found breeding in salt water in the Philippine Islands; they are *Culex microannulatus* Theob., *Myzomyia ludlowii* Theob., and *Culex* sp. indet. In a former publication [2] I stated that *C. microannulatus* Theob., breeds in brackish water, but it has since been found in the same places as *M. ludlowii* Theob.; that is, in water which upon analysis yields a greater percentage of salt than that of Manila Bay. *Myzomyia ludlowii* Theobald was the species used in the transmission of malarial parasites in the series of experiments recorded in this paper. It is quite evident from collections made in the various parts of the Philippine Islands, that this species is quite generally distributed. It has been taken in Pampanga Province, in Jolo, Panay, Negros, Cebu, Manila, Cavite and Mindoro, so that its range is probably general in this Archipelago. It is always found in what we might term tidal backwaters, namely in swampy regions where the incoming tide floods considerable areas intersected by channels and dikes. In these places a certain amount of water is left by the outgoing tide, and as evaporation takes place, the remaining water becomes more salty until the next tide, when it is once more restored to nearly its ordinary specific gravity.

The amount of rain during the season when these mosquitoes are most abundant, namely from November to July, is not sufficient to render the water which remains in their breeding places sufficiently fresh to injure or destroy the plant life upon which the mosquitoes feed, or to be detrimental to the insects themselves; on the other hand it has been conclusively proved that they can not live in fresh water, nor in salt water which has been brought up to the point of saturation. Certain species of algæ are always found in salt water where *Myzomyia ludlowii* Theob., breeds. The commonest of these forms are *Enteromorpha* sp. indet. and *Chaetomorpha* sp. indet.[3] and the cells of these plants are found in the mosquitoes' stomachs. The insects are top feeders and make little or no use of the slimy algæ which grow upon the muddy bottoms of the pools or ditches in which they live, unless the water has so far receded as to bring these plants to within half a centimeter of the surface. Few other water plants are found in the breeding places of *Myzomyia ludlowii* Theob., but these, where they occur in small quantity, do not appear to affect the larvæ. The fact that none of the latter are found in water the surface of which is covered more or less completely with leaves of aquatic plants, goes to prove further that these insects in the larval and pupal stages thrive best under the almost uninterrupted glare of the sunlight, and that they retire to the shaded places only at the time of metamorphosis, when they remain below the surface for considerable periods before they came to the top to breathe.

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[2] *This Journal* (1906), **1**, 988.

In laboratory breeding experiments the plants in the water begin to die within three to five days, while the larvæ appear to feed in a desultory manner. The time for their natural transformation to the pupa comes and goes and they still remain as larvæ. The foulness of the water, due to organic decomposition, appears not to affect them, but on the other hand, the lack of proper food seems to cause them to remain in an indefinite larval stage, until after three weeks or more they gradually begin to die. Pupæ brought in and kept under similar conditions develop normally and the mosquitoes emerging from them appear not to have suffered from lack of sunshine in their previous stage. The conclusions are, therefore, that direct sunlight is absolutely necessary for the best development of this mosquito.

In order to obtain a sufficient number of individuals for experiments in biting, a large, white, gauze net was built over a pond the area of which was approximately 7 square meters. This net was high enough so that a man could enter by stooping down, and go from one part of the inclosure to the other on boards about 15 centimeters above the surface of the water. The earth banked up inside and outside the net held it in place and kept the mosquitoes from creeping out beneath the flaps; it also served as a resting place for the newly emerged adults. They were never found upon the net itself, but always upon the earth, and being so nearly of the same color it was not an easy matter to find them.

While there was no appreciable difference in the appearance or rate of development of the larvæ, the adult mosquitoes were of a slightly paler color when they were reared under nets and for some reason appeared to be less active or wary than those individuals encountered in the open.

Care was taken in constructing the net to exclude all small fish that might be enemies of the mosquito larvæ, so that when masses of algæ containing large numbers of mosquito eggs were placed in the water, the insects could develop without menace from this source. However, it is probable that a limited number of the larvæ and pupæ fell a prey to the tiny water beetles and their larvæ, which could not all be removed from the water owing to the fact that many of them burrowed in the mud when the pond was disturbed. One or two dragon-fly larvæ were left in the water and they reached maturity. They were seen to capture some of the hundreds of *Myzomyia ludlowii* Theob., and *Culex microannulatus* Theob., which emerged, but their quota must have been very insignificant, since they were small damsel-flies and not the larger and more voracious *Libellulinæ*.

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[3] I am indebted to Dr. W. R. Shaw, Philippine Normal School, for these identifications.

One of the most satisfactory methods of collecting the adult mosquitoes for experiment was to permit a large number of the larvæ to pupate under the net among the algæ and, within a day of the time for their emerging as adults, to collect as many as needed with a scoop net. They were then placed in a jar of salt water and covered; the next morning all of the adults would be found, they having emerged during the previous evening.

Many attempts were made to raise these mosquitoes in confinement, but I succeeded only after numerous trials. Under ordinary conditions evaporation is so rapid that water must be added each day; this so disturbs the larvæ that they do not thrive well, especially when an attempt is made thoroughly to mix the water at the time of its addition. Another detrimental feature is the amount of reflection of light from the sides of the vessel. Thermometric tests show that the air just above the water contained in a 10-liter porcelain evaporating dish is from 5° to 7° C. above that of the outside. In 20-liter cylindrical glass vessels of 35 to 40 centimeters height it is from 8° to 10° C. Direct sunlight does not detrimentally affect the larvæ and pupæ, but the adults die within fifteen to twenty minutes when so exposed, so that those emerging under such conditions in a covered vessel never reach the stage where they can fly. Those not killed directly by the sunlight become so thoroughly soaked by the moisture collecting on the sides of the vessel that they soon die. It is possible, by using a large porcelain evaporating dish and shading a portion of it with a black cloth under white, to keep the temperature down 2° or 3°, but even under such conditions great vigilance and a repeated change in the position of the vessel to a shady place for an hour or so and then back into the sunshine, is necessary.

LIFE HISTORY OF MYZOMYIA LUDLOWII THEOB.

The egg.—One and eight-tenths millimeters in length and 0.95 millimeter at its widest point including the air-cells, it being slightly wider than deep. The egg (Pl. I, figs. 1 and 2) is nearly jet black, the surface being covered with an iridescent, reticulated film which separates when the egg dries. It is blunt canoe-shaped, the lower surface strongly convex, the upper concave lengthwise. At each end is a small, round, lighter area having 7 to 8 tiny black dots within its circumference. A rim of air-cells extends around the entire upper part and at each side near the top and midway between the extremities occupying nearly one-half the length is an arrangement of air-cells which extends from the top halfway down the side of the egg (Pl. I, fig. 2). These cells run dorso-ventrally, each one widening downward.

The eggs are usually laid in the afternoon after 4 o'clock. When ovipositing the insect alights upon the water at a place where a mass of algæ exists. After walking around for a brief time, it finally extrudes the eggs one after another, at the rate of about one per second until five or six have been laid; it then walks a short distance away and then deposits a few more. It has thus far been impossible to determine the number laid by a single female, but counts of the eggs found in the ovaries and oviducts of new females show it to be seventy-five to one hundred and fifty, the average being about one hundred.

Larva (Pl. I, fig. 3).—The larvæ hatch in from thirty-six to sixty hours from the time of oviposition, the period of incubation depending upon the temperature.

The egg shell in hatching splits longitudinally on the upper surface, somewhat in the manner of the eggs of Muscidæ, the small piece breaking out and the shell afterward shriveling and rolling up. The young larvæ are pale gray, almost transparent and have a white spot on the anterior area of the thorax. The head is marked by a prominent, black band extending around the entire posterior margin and there are a few dark brown markings on its surface. The dark, triangular spots before the eyes persist throughout the larval period and are the beginnings of the adult compound eyes. This may easily be seen just before the last molt and when the pupa has formed within the larval skin.

The full grown larva (Pl. I, fig. 3) is greenish-gray, the abdomen is dorsally of this color, slightly darker near the median line. The region around the anterior dorsal and ventral portions of the thorax has a decidedly blue color subcutaneously. There is a small, transverse, black tergite between every two abdominal segments. These tergites appear at first sight to be the result of great transparency and the consequent visibility of the stomach and intestinal contents, but they are of course cutaneous. The ventral surface of the thorax and abdomen is pale bluish-green-gray.

The head is light buff, with the following dark brown markings above: The small, round eyes at the lateral prominences; a large subtriangular band, representing the compound eyes of the adult in front of each eye; a narrow band around the posterior rim of the head; a transverse, broken, wavy band five-eighths the distance across the top, before the eyes; a V-shaped line medially, opening anteriorly and connected posteriorly with the band on the posterior rim of the head; a small, triangular, median dot between the legs of the V; a small, round dot behind the eye, halfway from the posterior margin.

On the underside, the head is marked as follows: A large, brown patch on the ventro-lateral area posteriorly; a furcula-shaped mark, its apex at the mouth, extending posteriorly nearly to the margin; a small dot at the basal angles of the labium. The tips of the mandibles are black. (Pl. I, fig. 5.)

The labial plate is of peculiar structure, being bilaminate, the proximal lamina has 9 teeth, the external lamina 5. A thin, chitinous prolongation of the cusps extends into the head and serves as a brace. (Pl. II, fig. 4.)

The labium is composed of a movable portion which has 15 very stout, blunt, curved bristles along its margin and is covered with smaller, scale-like processes extending also to the clypeus. (Pl. II, fig. 2.)

Dorsally on each of the second to sixth abdominal segments, midway between the median line and the lateral angle, is a small tuft of stellate hairs, used for supporting the larva horizontally beneath the surface film. When it comes to the surface these hairs spread out, engaging the film. Each abdominal segment has laterally one or two pectinate bristles, diminishing in size toward the anal extremity.

Chœtotaxy of the full grown larva.—In order to avoid confusion the arrangement of the hairs on one side of the median line is given.

Head (Pl. II, fig. 1).—At the base of the clypeus, projecting anteriad over and to end of the brush, is 1 straight, simple bristle, slightly to one side of the median line; postero-mediad to the base of the antenna 1 straight pectinate bristle and 2 more in a transverse line mediad to it and equidistant; exteriad and ventrad to the antenna, curving anteriad, is 1 pectinate bristle; ventrad to this is a group of 3 short, straight hairs of equal size projecting from the same point; posteriad to this another group of 3, antero-ventrad to the simple eye and slightly remote therefrom; anteriad to the median angle of the compound eye-spot is 1 simple bristle projecting at a right angle to the surface of the head; there are no hairs or bristles on the ventral surface. The maxillary palpus is armed with a compound, palmate-pectinate bristle at the outer side of the apex, before the articulation of the terminal joint. (Plate II, fig. 3.)

Thorax.—On the anterior face (Pl. III, fig. 2) laterad to the point of articulation of the head is a very small, bi- or tri-furcate bristle projecting anteriad; 2 long bristles in a transverse line on the anterior area of the dorsum, laterad of the median line; laterad of the exterior of these and distant from it three times its distance from the interior bristle, is a short, stout, pectinate bristle projecting anteriad; exteriad to this is another, more slender, nearly twice as long, projecting parallel with it, the latter followed laterad by a slender, simple bristle of its own length, projecting latero-anteriad; latero-ventrad to this is a pectinate bristle nearly twice its length, projecting latero-anteriad; ventrad of this is a curved, pectinate bristle projecting anteriad on the anterior face of the thorax; at the intersection of an imaginary transverse line through the middle of the thorax and one a little more than midway between the median line and the dorso-lateral margin, is a long, pectinate bristle projecting antero-laterad; laterad of this are 2 small, simple bristles and exteriad to these, 4 others, the most ventral of which is quadrifurcate; on the posterior lateral or metathoracic area are 4 curved pectinate bristles, all twice the length of those on the anterior or prothoracic area of the thorax. These bristles are all movable by means of voluntary muscles. The most ventral bristle has a small pseudopod at its origin. (Pl. III, fig. 3.) The same is true of the most ventrad of the anterior or prothoracic bristles and of a similar one medio-latero-ventrad on the mesothorax.

When a larva is examined ventrally it will be seen that the pseudopoda occupy a position relative to the six feet of a coleopterous or lepidopterous larva, a pair on the pro-, meso- and metathorax respectively. They are located on prominent, tumescent tubercles and are all capable of a slight motion in conjunction with the pectinate bristles. The middle and posterior pair are the only ones which show signs of articulation, each pseudopod being composed of two segments, not only visible in the living larva but also in the exuviæ and in macerated speciments. I am thoroughly convinced that these organs represent atrophied or degenerate organs of locomotion, not only because of their morphology, but also from their function, as observed in the living larva when it crawls over vegetation, or upon a rough, moist surface. I do not recall that any previous author has mentioned these organs and I call attention to them at this time in order to elicit a further discussion as to their origin and possible significance. I have noted the same organs in the larvæ of *Worcesteria grata* Banks and in those of certain other Culicidæ. I hope to present drawings of them in a paper dealing with the life histories of the Philippine mosquitoes.

Abdomen (Pl. I, fig. 3).—The *first* segment has a pair of lateral, approximate, pectinate setæ, curving forward as on the thorax; ventrad to this is a tuft of 3 simple setæ, submediad on the posterior area of the ventrum is a tuft of 3 short, simple setæ; the *second* segment is as the first, except that on the sublateral, posterior area is a tuft of 3 to 5 palmate hairs, laterad of which are 3 short, erect setæ in a transverse line; the *third* segment has a single, lateral, pectinate seta, a palmate-hair tuft consisting of 11 to 12 parts, 3 short setæ on a line anteriad of the palmate tuft and a small tuft of simple setæ posteriad of the pectinate seta, in addition to the ventral tuft; the *fourth* segment has a small, pectinate seta laterad and a subsidiary tuft of 3 to 4, simple setæ slightly posteriad. The palmate tuft on this segment contains 14 hairs and antero-laterad of this is a simple, erect seta, four times the length of the hairs in the tuft; the *fifth* segment is similar to the *fourth*, but its palmate tuft contains only 8 hairs; the *sixth* segment has several pale, scattering, simple setæ on the latero-dorsal area posteriad. Its palmate tuft is composed of 10 to 12 hairs. The *seventh* segment is similar to the *sixth;* the *eighth*, bearing the respiratory siphonette (Pl. I, fig. 6), has a few scattering setæ laterad. The *ninth* has a quadri-pectinate bristle, laterad, near its apex, and a series of 9 very long (twice the length of the segment) pectinate hairs, curving at their tips and articulated on a special, movable tubercle (Pl. I, fig. 6), the whole serving the double purpose of rudder and propeller in the locomotion of the insect. At the extreme caudal end of this segment, dorsad to the anal aperture are 4 palmate, brush-like setæ, the ventral pair of which has coarser branches than the dorsal, their number being 18 (in 9 pairs) and their extremities being curved. They are used by the insect for anchoring itself to plants on the surface of the water. The whole dorsum of this segment is covered by a single, chitinous sclerite (Pl. II, fig. 5) from the middle of each side of which projects a pair of simple, erect setæ.

The tracheal gills (anal gills) (Pl. I, fig. 6) are very short as compared with those of *Culex* and *Stegomyia* larvæ, which remain a long time beneath the water. These gills lie immediately around the anus and are about twice as long as broad.

The length of the larval period is from nine to thirteen days from the hatching of the egg to the transformation to the pupa. During this time the larva molts five times, the first stage being one and one-half to two days, the second two to three, the third two to three, the fourth two to five and the fifth two days.

The respiratory siphonette (Pl. I, figs. 3, 4 and 6).—This organ, situated on the posterior, dorsal area of the *eighth* segment, which is obliquely truncated to receive it, is of very peculiar structure and merits a detailed description. When open (Pl. I, fig. 4) it has the form of an irregular, elongated five-pointed star, the lobes of which are rounded. The anterior lobe is the largest and lies mediad. It is thin, mica-like in appearance, hinged at its base, and has a vitelline, globular tubercle at the median point of the base. This vitelline tubercle fits into a socket when the lobe is closed over the two tracheal apertures, each of which lies a little to one side of the median line. When the organ is closed, the two remaining anterior lobes fit against the respective posterior lobes, the latter doubling outward along their axes and inward along their points of union with the anterior lobes. The four lateral lobes close before the median, which fits against the others, effectually locking itself by means of the tubercle and socket and precluding the entrance of water. The entire structure is reënforced with strips of chitin running through various parts as shown by the darker lines in the figure. On each side of the siphonette is a lateral comb (Pl. I, fig. 6, and Pl. II, fig. 6) composed of fifteen teeth, those ventrad being longest and all pointed directly caudad. The comb is surrounded ventrad, anteriad and dorsad by a black, chitinous brace, from the dorsal cusp of which projects a quadrifid seta (Pl. I, fig. 7) four times the length of the comb teeth.

Pupa (Pl. IV, fig. 1).—The color of the pupa is dull gray, with a slightly greenish tinge. The last abdominal segment is light ocher. The first abdominal segment is darker dorsally than any succeeding one. The respiratory siphon is dark gray externally, the inner surface being pearly-white and striate. The eye-spots of the adult mosquito are plainly visible as dark areas on the antero-lateral region of the pupal cephalo-thorax.

The chætotaxy of the pupa is as follows, the hairs and bristles of but one side being given as in the larva: Submediad on the anterior margin of the metathorax is 1 small, simple erect hair, on the scutello-abdominal suture of the *first* abdominal segment, submediad, is a short, erect, bifurcate bristle, followed laterad by 2 simple bristles, the second of which is 3 times as far from it as the first; submediad on the middle of the *second* abdominal segment is a palmate tuft of hairs composed of about 30 simple and bifurcate branches; submediad on the *third* segment at its middle is a quadrifurcate bristle, followed laterad by a simple, stout, curved bristle; on the *fourth* segment is a long, trifurcate bristle, submediad on the posterior margins of the tergite, followed laterad by two other simple bristles, the first of which is anterior to the margin, the second on the margin; on the *fifth* segment there is a bifurcate, submedian bristle, followed laterad by a trifurcate bristle and antero-laterad by a bifurcate, smaller one; the *sixth* segment has a simple, submedian bristle, longer than the segment, on the posterior margin of the tergite, followed laterad by a quadrifurcate bristle, the *seventh* and *eighth* have bristles arranged similarly to those on the *sixth* except that on the *seventh* there is a very small hair, mediad of the long bristle, and its lateral bristle is bifurcate; on the *eighth* segment there is a trifurcate bristle anteriad of the long, simple bristle; the *ninth* segment bears a stout, dark brown, slightly curved bristle at the posterior lateral angle and a single, submedian, dorsal hair at the posterior margin.

Ventrally the *fifth* and *sixth* segments have a stout, latero-ventral bristle at their posterior margins, while on the *seventh* and *eighth* there is a submedian bristle on the posterior margin.

The caudal fins, or pinnura (Pl. IV, figs. 4, 5), have the central and external marginal veins brown at their bases, while the prolongation of the central veins, the urachætæ, are long, slender and recurved at their apices, forming a hook (Pl. IV, fig. 5). The external margins of the pinnura are fringed with delicate hairs from the urachætæ to the apex of the external veins.

Length of pupal period.—The pupal stage lasts from two and one-half to three days according to weather conditions.

Habits of pupæ.—The pupæ, as a rule, remain at the surface in full sunshine, but swim very rapidly to the bottom or among or beneath the layers of algæ when any object comes near. They can remain submerged for from fifteen to forty-five seconds, but usually return to the surface almost immediately. Their pale gray color makes them very difficult to detect, and were they to remain quiet at the surface they would be well protected by the background of green or light gray of the decaying, matted algæ. No special or notworthy difference appears to exist between their position and that of the pupæ of *Culex microannulatus* Theob., with which they are often intimately associated. A mass of congregated pupæ will frequently contain individuals of both species in about equal number and the pupæ of *Myzomyia* are only distinguishable by their lighter color and slightly smaller size.

Myzomyia ludlowii Theob.

Description of adult female[4] (Pl. V, fig. 1).—Palpi deep brown, the apex broadly white-banded, another small band close to it and a third much lower down (Pl. VII, fig. 2); proboscis deep brown, with distinct, creamy-white tip. Thorax fawn-colored in the middle, dark brown at the sides, with a median and lateral dark lines and curved, hair-like, pale scales; abdomen brown, with pale hairs. Legs mottled and spotted with yellow; tarsi apically and basally pale, banded. Wings with 4 large costal spots and one or two small basal ones, most of the veins of wing area pale scaled. (Pl. VII, fig. 3.)

♀ Head pale brown, with narrow, pale scales and pale and brown, upright, forked ones, a pale, median tuft projecting forwards; antennæ brown, basal joint pale ferruginous; proboscis deep brown, apex creamy, palpi deep brown, with a broad, creamy, apical band, and near it another narrow, pale band, the remainder divided by another narrow, pale band, base densely dark scaled.

Thorax fawn-colored in the middle, dark brown at the sides, with a median, dark line and a narrow, dark line on each side of the pale area, with scanty, hair-like, curved, pale scales and traces of a dark spot before the scutellum, which is pale brown, with narrow hair-like scales; pleuræ brown, mottled with gray.

Abdomen brown, with narrow, curved, hair-like scales and pale posterior border-bristles.

Legs brown, the femora and tibiæ and metatarsi, especially in the hind legs, spotted with yellow; tarsi with broad, apical and basal, pale banding, especially on the hind legs; ungues small, equal and simple.

Wings with 4 large, dark, costal spots and two small, basal ones, the apical spot small, extending evenly on to the first long vein; this is followed by a pale area nearly twice as long as the black apical spot. The second black spot is about the same length as the preceding pale one, and spreads evenly on to the first long vein; the next pale area is slightly longer. The third black spot is the largest and spreads nearly evenly on to the subcostal vein, while beneath it on the first longitudinal is a large, black line and then a small, pale area followed by a small, black spot, the black line not beginning directly under the costal spot. The fourth black spot is separated from the third by a very small, pale area, and extends evenly on to the subcostal and first longitudinal; at the base is another small, black spot. The second long vein has dark scales on each side of the cross-vein and a dark spot on the upper branch of the fork-cell under the apical costal spot, and another small one near its base; the lower branch has an apical spot and a larger one near its base. The third long vein has a black, apical spot and a dark patch on each side of the cross-veins; the fourth is mainly dark on each side of the cross-veins, and has 2 dark spots on the upper branch, one near the base and two on the lower branch; the fifth has a black spot at its root, 3 on the upper branch and one at the apex of the lower branch; the sixth has 2 dark spots. The first submarginal cell is a little longer and decidedly narrower than the second posterior cell, its base slightly nearer the apex of the wing than that of the second posterior cell, its stem as long as the cell; stem of the second posterior considerably longer than the cell; supernumerary and mid cross-veins in one line, the posterior rather more than twice its length distant from the mid, but very variable, sometimes step-like; fringed with pale spots.

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[4] This description of the female adult (Pl. V, fig. 1) is taken from Theobald, *Mono. Culic.* (1903), 3, 42, while that of the male is prepared by myself, this sex having never been described.

Length.—4 to 4.8 millimeters.

Habitat.—Luzon, Philippine Islands (Miss C. S. Ludlow).

Time of capture.—April.

Observations.—Described from a number of specimens. A very variable species, somewhat like *Rosii* at first sight, but easily told by the spotted legs and much shorter fork-cells. The base of the first submarginal is always slightly nearer the apex of the wing, and the costal spots differ slightly, but are to some extent variable. The cross-veins are most unstable. The palpi are very similar, but the apical band in *Rossii* is rather longer. The chief difference is that in *Rossii* the second white band is a third of the way down the palpi; in this species it is less, and the black, intervening area is much smaller.

Description of adult male (Pl. V, fig. 2).—Pale-gray, the disc of the thorax being lighter than other parts of the body, the abdomen and pleuræ of thorax darkest. Head with dark-brown, erect scales at sides and white ones in middle of occiput. Eyes ruby-red, with curved bristles projecting over them anteriad from their posterior margins; frontal tuft long, cristate, prominent, antennæ pale golden-brown two distal segments dark-brown; the 3-jointed palpi long, slender, except the two apical segments which are tumescent (Pl. VII, fig. 1), with rounded apices and the penultimate having an internal tuft of long, golden hairs at its base; basal $\frac{2}{5}$ of first papal joint dark-brown, followed by narrow, white band; apical $\frac{3}{5}$ or less, dark-brown with a long, narrow, white spot covering its middle third and a transverse, triangular, white spot at its apex. The second segment all white above, except a narrow, brown area at its base, the entire under surface being brown also; the apical segment nearly all white, except a very narrow area of brown at its base, the proboscis dark-brown, except the tip which is lighter. The palpi and proboscis are of the same length. Around the lower rim of the eyes is a fringe of dark-brown hairs, pointing posteriad.

The denuded thorax shows one median and one submedian, thin, dark line on anterior $\frac{3}{4}$, the remaining surface being covered with a whitish pruinescence.

The costæ of the thorax are longitudinally striped dark brown and gray, there being approximately 13 or 14 stripes. The prothoracic lobes are well defined and bear a tuft of dark-brown setæ. The scutellum is perfectly nude, gray and with a transverse, median, dark brown spot; the meanotum nude, dark gray. Halters pale gray with brown knobs.

The abdominal segments uniformly dark mottled-brown with the entire surface uniformly decorated with fine, golden setæ. On the eighth segment and the harpes dorsally the surface is covered with white scales, in addition to the abundant, golden setæ.

The wings are as in the female, except that they are paler.

The legs are uniformly golden and brown mottled, the apices of the tibiæ, metatarsi and tarsi being pale, banded, especially the posterior tarsi which are themselves darker than the remainder of the legs.

The first anterior tarsal joint has a double row of short, stout, dark spines on its entire lower surface, there being about 108 in alternate succession. The empodium is $\frac{1}{4}$ the length of the fifth tarsal joint, its median seta making its total length $\frac{1}{2}$ that of the joint. There is a small, blunt spur at its base.

The anterior tarsal ungues (Pl. VIII, fig. 1) are unequal, the larger being curved and having a single tooth at the middle.

Length of ♂ 5 millimeters, length of wing 3 millimeters.

Manila, P. I.

Time of breeding, 14 August, 1906.

Type ♂, No. 5744 in Entomological Collection, Bureau of Science, Manila, P. I.

Only the female of this species has been described previously by Theobald, and I find no mention of the male having been taken. All my males were obtained by breeding. They are about equally as abundant as the females in bred material.

I have noted that when at rest, the males fold their wings so that the costal edges instead of being parallel, as in the female, converge posteriorly, giving the insect a distinctly tapering appearance. This feature will be a sure guide to distinguishing the sexes in living specimens when they are at rest, and even when the palpi are not readily visible or distinguishable. The males feed readily on banana and suck large quantities of salt water, their abdomina often appearing almost transparent from the amount taken. The structure of the mouth-parts of the male is such that it is impossible for it to puncture the skin and therefore it can not bite.

HABITS OF THE ADULT.

Contrary to the general rule and to my expectations, this mosquito is essentially a dry-season form; that is to say, it is most prevalent during the months from November to June. I believe it to be a strictly salt-water form, delighting, during the larval and pupal stages, in the brightest sunshine. This explains its comparative scarcity in the rainy season. Its breeding grounds, because of the tides, never dry completely. The temperature during the dry period is very favorable to the multiplication of several generations. The lack of hard rains, which would beat the surface of the water in the breeding places, makes it possible for the greatest number of eggs to hatch and of larvæ to reach maturity, while the adults find sufficient shelter from the sun, which is fatal to them, in the dense foliage and along the banks of their breeding places.

If the adults are disturbed during the bright, sunny hours of the day, they invariably fly but a short distance, alighting in the characteristic attitude, always in the shade and usually upon the underside of a leaf.

These mosquitoes are frequently found in houses and their favorite resting places are the undersides of tables, cupboards, beds, in the dark corners of ceilings or upon the portions of dark clothing which are shaded. The females are the ones invariably encountered. It appears that the males never leave the vicinity of their breeding places.

I have observed that both sexes drink salt water when they are kept in confinement, usually within an hour after their emerging from the pupa.

Copulation.—The sexes are equally active and in the late afternoon they may be found copulating, both in the free state and in captivity. The mode of copulation is the same as that of *Stegomyia*. The females which hang from the leaf or the gauze over the breeding jars, loose their hold with their middle legs and hang only by the fore legs when approached by the males. The latter fly against them from the ventral side, entangling their legs with those of the female, the anterior pair being clasped around the thorax just back of the neck and the middle pair being sometimes apposed to those of the female, sometimes entangled. The act of copulation then takes place and may last as long as 30 seconds, the male flying away to another part of the jar and the female proceeding to preen herself after the manner of house flies. One male has been observed to copulate with four or five females within as many minutes. None of these mosquitoes have been observed doing so while on the wing, although it is not unlikely that this does occur.

Egg-laying.—The female when about to lay her eggs wanders on the wing just above a place where the algæ are partly out of the water. She settles down after a short time, walking somewhat rapidly over the surface, trying different places with the tip of the abdomen. During this latter process the body is about parallel with the surface of the water, while the head and palpi are inclined downward. This is apparently the only time in this mosquito's life as an adult when her head and proboscis are not in a line with the main axis of the body. When the ovipositor encounters a small particle either of the algæ or of other material projecting a short distance above the surface film, the female stops, palpates with the tip of the body and almost immediately oviposits, going thus from point to point till she has laid from ten to twenty-five eggs, she then stops for a time to preen herself and to rest. Sometimes as many as seven or eight eggs will be laid in a place. As the females which laid their eggs in captivity did so only after having been fed a meal of blood, I suppose that those observed in nature had also procured such food, although at the time of the observations their bodies showed no signs of having blood within them. This may be due to the fact that the eggs are laid some five or six days after the insect has fed. Those mosquitoes which were observed laying in captivity were seen to have white masses of eggs within the abdomen posteriorly, just before they were observed to begin ovipositing.

Habits of the female.—The female is much more active than the male after copulation, which occurs after thirty-six hours of emergence. Although she remains quiescent during the day, she is very alert after 5 o'clock in the afternoon. In both sexes the position of the body is even more pronounced than that shown in drawings and sketches of Anophelinæ by various authors. In some instances individuals are seen which have the body nearly at a right angle to the surface upon which they are at rest and the posterior pair of legs is bent up over the back so that the tarsi are not very remote from the dorsum of the thorax. The female when disturbed flies off very rapidly in a straight line, and if annoyed when in the act of inserting her proboscis for biting, she almost invariably returns to the same point to renew the attack. When resting upon a wall or other object the females of *Myzomyia ludlowii* Theob., are not difficult to capture in very small vials, provided the extended hind legs are not touched. These limbs are evidently intended to serve the same purpose as the rostral hairs of animals like tigers, cats or mice. They are kept in almost constant motion, sometimes one is elevated, more frequently both. The males are somewhat more wary than the females.

Blood-sucking.—The females of these mosquitoes attack readily in the evening; during the day it is not easy to persuade them, even when the receptacle containing them is covered with a black cloth. When one of the insects settles upon a chosen spot, its palpi are immediately elevated and the stylets of the proboscis inserted, sometimes to within less than a millimeter of their base. During the process of inserting the stylets, the female wriggles her palpi in a manner which seems plainly to suggest the pleasure which the act affords. After she has begun to draw blood, the motion of the palpi decreases somewhat, but does not entirely cease until she has completed the meal and withdrawn her proboscis.

An invariable habit of these mosquitoes while feeding on blood for the first time is that of voiding a serum-like liquid from the anus in a continuous stream of droplets. This is succeeded by pure blood with the corpuscles intact. An amount equal to that retained in the body will frequently be expelled in this manner in five or six minutes from the time the insect begins to bite. These dejecta retain all the microscopic appearances of uningested blood, excepting that the corpuscles are fewer in number, the greater part having been retained, probably in the stomach. All dejecta, subsequent to this peculiar one, are of the normal flyspeck kind, and contain the usual waste products of digestion. It appears as if the first meal of blood which the insect takes, acts as a very rapid cathartic and prepares the system for the digesting and assimilating of succeeding meals.[5] Unfortunately, no observations were made upon mosquitoes fed, previous to sucking blood, on the juice of bananas.

Susceptibilities and idiosyncracies.—Female mosquitoes of this species stand confinement very much better than males, the latter dying in from two to four days after emerging, even when abundantly supplied with food which they appear to enjoy. The females may be kept alive for at least twenty-three days by feeding them on blood every alternate day. It has been stated that unmodified sunlight is fatal to the adult mosquito, but the males succumb to such conditions very much more rapidly than the females. They are in every way more fragile than the other sex. They have never been seen to gorge themselves, either with juice from ripe bananas, water or sugar sirup, as do the females, and of course they *positively* do not suck blood. This has been thoroughly demonstrated by trials with more than five hundred male mosquitoes under every condition which could arise. If they can be directed to a drop of blood placed on the side of the vessel containing them, they will sip it up daintily for a short time, but never remain at it, as do the females, until the last bit has been drawn up.

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[5] It may be that the saline character of the blood gives it this cathartic effect.

MOSQUITOES AND MALARIAL PARASITES.

The examination of some five hundred blood smears from about fifty patients in the "naval sick quarters" at Olongapo demonstrated the presence of both tertian and æstivo-autumnal parasites, the former comprising about 65 per cent of the cases, the latter 35 per cent. In no instance was the parasite of the quartan infection positively identified, either microscopically or clinically. Abundant material was therefore continuously at hand for purposes of experimentation, and there was not a single case in which any of the men objected to allowing themselves to be bitten as many times as I desired. Many of the men were examined only once, but a number had fifty to sixty slides made from their blood in various stages of the development of the parasite and several of them were bitten by not fewer than twenty-five to thirty mosquitoes, this, of course, including some who were bitten as often as twelve to fourteen times by the same insect.

METHODS OF BREEDING.

Two methods were used for obtaining the adult mosquitoes for purposes of biting, one being to construct a cage over the breeding pond and to take the adult females during the early morning; the other to collect many of the mature pupæ at about 4 o'clock in the afternoon and to wait for the emergence of the insects on the same evening, which in the great majority of cases took place between 6 and 8 o'clock, although in fewer instances the time was retarded until the next morning. It was found that those emerging at eventide were ready and willing to bite by 9 or 9.30 o'clock at night, but that those which appeared in the early morning seldom were ready to attack until the succeeding night after 6 o'clock.

As soon as the adult mosquitoes emerged and had dried sufficiently to be able to take flight, they were removed from the breeding receptacle directly into glass tubes 16 centimeters long and 4 centimeters in diameter, over one end of which were fastened, by means of rubber bands, pieces of plain gauze. Wads of absorbent cotton were pressed into the other ends of the tubes, but not too tightly to prevent a circulation of air. When in the tube, the mosquitoes generally rested upon the gauze and this was sprinkled daily with water, which the insects drank each time it was applied. I attempted to observe whether the insects would copulate in the tubes, but have never seen them do so, although eggs developed in one or two females, so that fecundation had probably taken place. In placing males and females into the tubes, care was taken to select only those females which had not copulated in the breeding jar. They were carefully watched during emergence and removed, in some cases, even before the integument had dried. None of these mosquitoes were used for breeding purposes.

INOCULATION OF MOSQUITOES.

At the beginning of the work numbers of mosquitoes were fed upon blood from persons negative for the malarial parasite, and I also allowed several of the insects to bite me. The mosquitoes were dissected at intervals of from eighteen hours to several days after biting, for the purpose of becoming perfectly familiar with the appearance of the internal organs, including the stomach, the thoracic cavity, muscles, and the salivary glands under normal conditions. After this had been done and the technique thoroughly outlined, patients were selected whose blood had, upon examination, shown the greatest number of parasites of the æstivo-autumnal type, which was chosen because of its great prevalence among the troops and the presence in the hospital of men who at the time were well infected by the crescent forms. The tubes used to carry on the work were prepared so that some contained a single insect, others held six and others twelve; those with but one being kept for voluntary cases who would submit to being bitten by the infected mosquitoes. The others contained insects which were to be allowed to bite malarial persons and then were to be placed in alcohol or dissected at various periods in the development of the parasites.

Dissection of mosquitoes.—Six hours after the mosquitoes had first bitten the patient, the insects were dissected and, from the blood within the ventral reservoir, smears were made upon slides, fixed and stained with Wright's stain after the usual method. At first some difficulty was experienced in obtaining a satisfactory adhesion of this blood to the slides. It was found that by either heating alone, or fixing with absolute alcohol and ether alone, the blood would largely be removed when it came to staining the slide. Finally, a combination of the two processes was used, heating first and treating with alcohol-ether afterwards being resorted to.

The crescents and ovoids were demonstrated in the blood obtained as above, but, unfortunately, mosquitoes in this stage were not placed in alcohol, so that no specimens of this part of the phenomenon were preserved except on the smears of blood taken from the stomachs of the insects.

Mosquitoes were dissected daily from the time of the first ingestion of blood, but nothing of interest was noted in the unstained tissues up to the day when the swellings in the gastric mucosa indicated the probable growth of the oöcysts. The fourth and fifth days' dissection demonstrated a considerable enlargement of the oöcysts, which attained a diameter of from 30 to 60 μ. On the sixth and seventh days they were for the greater part found as collapsed bodies on the cœlular surface of the stomach wall.

Sectioning of mosquitoes.—Further tracing of the parasites in any but sectioned specimens was impracticable. Mosquitoes were therefore put into alcohol and sent to Manila for sectioning, so that all stages of the passage of the parasites to the salivary glands might be traced. By this means their presence in all the intervening tissues between the stomach and the salivary glands was demonstrated. The accompanying photomicrographs (Pl. IX, figs. 2, 3, and 4, and Pl. X) show them also in the interstices between the muscle-fibers of the thorax, and in the salivary glands themselves.

Appearance of the sporozoites.—Specimens stained with hæmatoxylin-eosin, prepared according to the ordinary formula, show the normal muscular, glandular, and connective tissues stained red or blue, while within the cell structure itself innumerable, extremely small granules, either globular or elongate, appear, measuring not more than 0.75 μ in diameter and of a dark brown color. They are not only found within the cells of the tissue, but also, as is shown by certain salivary glands examined, adherent to the external walls of the gland cells. This phenomenon is shown in Plate IX, fig. 4, at the point marked *spor.*

TRANSMISSION OF THE PARASITE.

Upon going to Olongapo, it was not my intention as it was not my hope to procure volunteers who would be willing to submit to being bitten by mosquitoes infected with malarial organisms, so that I said nothing except in a suggestive way to the gentlemen in charge of the medical work at this station, until I had reached to the stage of demonstrating the oöcysts in the stomach wall of the mosquito. Finally I obtained one volunteer, the result being as follows:

CASE I. *Volunteer malarial infection.*—J. C. B. had been in the Philippines nearly two years; his medical history during his six years' service in the Navy gave no indication of his ever having had malaria. His statements were also to the effect that he had never had malaria, chills and fever, or any illness that would point to his having been infected. Many examinations of his blood demonstrated continuous absence of malarial parasites.

The patient was first bitten by a malaria-infected mosquito at 5 p. m. of June 1, 1907. This mosquito did not draw any blood, after having repeatedly thrust its proboscis into the patient's arms at different points. On the morning of June 2 at 8 he was again bitten by the same mosquito and by two others which were confined together in another tube. At 10.30 on the morning of June 3, at 10.30 a. m. of June 4, and at 8.30 a. m. of June 6 he was rebitten by the same three mosquitoes, they having had no other alimentation beyond that furnished by his blood. Every day, except on June 8, he showed a slight rise of temperature, but never above 37.4° C. On June 9 the patient complained of malaise, severe headache and chill, which latter was not very marked. Temperature at 12 o'clock, 38.3° C. In the evening the patient again felt well and during the next day his temperature was practically normal. On June 11 the temperature rose to 38° C., the patient having a slight chill, although he did not lie down nor desist from his routine work. From June 11 until the time of my departure, he had no further rise of temperature and no chill; he, however, continued his chart up to and including July 6. It shows that on June 24 he had a slight chill and that on the next day his temperature was 37.7° not rising again until June 28 when it reached 38° C., and again on July 2, 38.2° C. On July 1 the patient had headache, nausea, and general listlessness, with loss of appetite and frequent dizzy spells. On September 4 he reported as follows: "I had a spell of fever commencing with August 3 to August 14. I kept track of my morning and evening temperatures * * * noting them down in my * * * journal The afternoon of August 3 I had a chill and a temperature of 38.5°, then around 37.2° and 38.3° C. until August 6 when I had another chill and a temperature of 39.2° C., then fever off and on till the 14th of August."

Examination of blood from the patient.—On June 3 three slides were taken at 8 o'clock. Transparent, motile amœboid bodies moving freely from one red corpuscle to another were observed in a glass cell. They were evidently parasites which were too debile to enter the corpuscles. Nothing was noted on June 4. On the morning of June 5 the same bodies were seen in the blood serum. They appeared to move with greater vigor. The eosinophiles had attached large numbers of these bodies to themselves and in many instances they had engulfed them. On June 6 a stained slide gave a blue body within the pink corpuscle, but its identification was not certain. On the morning of June 7 the first positive identification of a parasite was made. It was half grown, of the æstivo-autumnal type. It was moving very slowly around an axis without appreciably changing its shape. The nucleus was plainly visible when stained and pigmentation had just begun. Another slide was taken on the same morning and stained by Wright's method. It demonstrated several parasites in the half-grown stage. On June 9 the parasites were again positively identified, also on June 10 to 12, but not on the 13th. On June 14 parasites were observed in the presegmenting stage, and on June 15 and 16 they were seen in all stages up to that of presegmentation. On June 17 a perfect æstivo-autummal pre-crescent ring was found, as also on the 18th, which was the last day of taking the blood.

A noteworthy feature of these blood examinations was the great numbers of parasites that were included by the phagocytes. Parasites which were seen to attack a red corpuscle at one moment would be engulfed the next by an eosinophile, although the phenomenon of the parasite entering the red corpuscle was frequently observed.

It should be borne in mind that the patient was bitten by infected mosquitoes up to June 6, so that naturally his blood would present an increasing number of parasites in the peripheral circulation toward the latter part of the experiment.

CONCLUSIONS.

The work on transmission performed upon the case cited above was in every way of such a positive character, the after effects so marked and the previous history of the patient so well ascertained that there remains no doubt in my mind as to the validity of the tests made. However, it seems very desirable, in order to subject this question to further concise test, that a number of volunteers be secured to undergo, in properly constructed cages, an isolation period followed by inoculation by *Myzomyia ludlowii* Theob., and as soon as the season of abundance for these mosquitoes returns I shall carry out further experiments looking to this end. The results of the continuous experiment at Olongapo have been published at this time as it may not be possible to furnish further proof until several months have elapsed, and it was not thought desirable to delay the publication of the work so far done.

ILLUSTRATIONS.

Map of Olongapo, Zambales, showing United States naval station and surrounding region with breeding places where *Myzomyia ludlowii* Theob., was actually found, indicated by red cross lines.

PLATE I.

FIG. 1. Egg of *Myzomyia ludlowii* Theob., top view, showing micropyles. 1×25.
2. Egg of *Myzomyia ludlowii* Theob., side view, showing large and small air-chambers. 1×25.
3. Full grown larva of *M. ludlowii* Theob. 1×10.
4. Respiratory aperture of siphonette of larva, showing vitelline tubercle in center of upper ray with the corresponding socket near middle of star and tracheal openings in upper part on each side. 1×40.
5. Right mandible of larva, showing dentation and auxiliary bristles and palpuli. 1×240.
6. Eighth and ninth abdominal segments of larva, showing (*A*) anchor bristles, (*B*) respiratory siphonette, (*C*) lateral comb, (*D*) tubercle for propelling setæ, (*E*) anal gills, and (*F*) propelling setæ.
7. Quadrifurcate seta from comb, the type of numerous setæ distributed over various parts of the body of the larva. 1×240.

PLATE II.

FIG. 1. Head of full grown larva, showing chætotaxy and markings. 1×60.
2. Labium of larva, with base of clypeus. 1×400.
3. Maxilla of larva, showing external, multiple seta and palpus. 1×400.
4. Ventral and lateral aspects of the labial plate of larva, the lateral aspect showing its double laminate structure. 1×400.
5. Dorsal chitinous sclerite of ninth abdominal segment of larva, showing paired anchor setæ (the outer ones) and propulsory setæ. 1×400.
6. Lateral comb from eighth abdominal segment of larva with chitinous brace and part of quadrifurcate seta. 1×400.

PLATE III.

FIG. 1. Head and thorax of larva in penultimate stage showing great width of colored, basal band on head. 1×60.
2. Dorsal aspect of thorax of full grown larva, showing chætotaxy. 1×60.
3. Ventral view of thorax, showing pseudopoda and chætotaxy. 1×60.

PLATE IV.

FIG. 1. Lateral aspect of pupa, showing chætotaxy and respiratory siphons. 1×12.
2. Lateral aspect of respiratory siphon. 1×25.
3. Dorsal aspect of respiratory siphon (1×25), showing opening lined with short, fine hairs which preclude entrance of water.
4. Dorsal aspect of abdomen of pupa, showing second to ninth segments and pinnuræ or caudal fins. 1×12.
5. Pinnura or caudal fin, showing form and position of urochæta. 1×25.
6. Perspective of abdomen of adult female mosquito, showing ovipositor and chitinous sclerites which compose the ninth segment. 1×60.
7. Perspective of abdomen of adult male mosquito, showing harpes and hairs together with sclerites which compose the ninth segment. 1×60.
8. Arrangement of supernumerary and cross veins in wing of male. (1×150): I. Supernumerary. II. Mid cross vein. III. Posterior cross vein.
9. Arrangement of supernumerary and cross veins in female. 1×150.

PLATE V.

FIG. 1. Adult female of *Myzomyia ludlowii* Theob. 1×8.
2. Adult male of *Myzomyia ludlowii* Theob. 1×8.

PLATE VI.

FIG. 1. Adult male of *Myzomyia ludlowii* Theob. Note position of claspers which is characteristic. This photomicrograph, as also the following, is made from a balsam preparation of a specimen treated with 5 per cent potassium hydroxide.
2. Adult female of same. Note the extruded spermatheca below the ovipositor. This organ lies normally within the eighth segment. The scutellum appears in profile as a knob at the base of the right wing.

PLATE VII.

FIG. 1. Eyes, antennæ, palpi, and proboscis of male *Myzomyia ludlowii* Theob. Note the construction at the middle of the second palpal segment, representing ankylosis of two segments.
2. The same of the female, showing the lancet and other punctorial setæ removed from the proboscis or labium.
3. Wing of *Myzomyia ludlowii* Theob., showing spots (female).
4. Wing showing arrangement of cross veins in the female.
5. Three ultimate abdominal segments of female showing spermatheca in position.
6. Eighth abdominal segment showing circi-like ovipositor with squamation.

PLATE VIII.

FIG. 1. Ungues of forefoot of male.
2. Ungues of forefoot of female.
3. Ungues of middle foot of male.
4. Ungues of middle foot of female.
5. Ungues of hind-foot of male.
6. Ungues of hind-foot of female.

PLATE IX.

FIG. 1. Genitalia of male of *Myzomyia ludlowii* Theob.
2. Section of thorax of infected mosquito, showing group of sporozoïtes of malaria at *A*. 1×1600.
3. Section of thorax of mosquito in vicinity of salivary glands, showing migratory sporozoïtes. 1×1600.
4. The lobes of salivary glands of mosquito with sporozoïtes thickly congregated around the upper one of them at *spor*. 1×1600.

PLATE X.

FIG. 1. Section of salivary duct of mosquito, showing agglomeration of malarial parasites with meganucleated cells in upper right. Best group of parasites at *A*. 1×1600.
2. Section of thorax of mosquito, showing sporozoïtes migrating through intermuscular spaces. 1×1600.

PLATE XI.

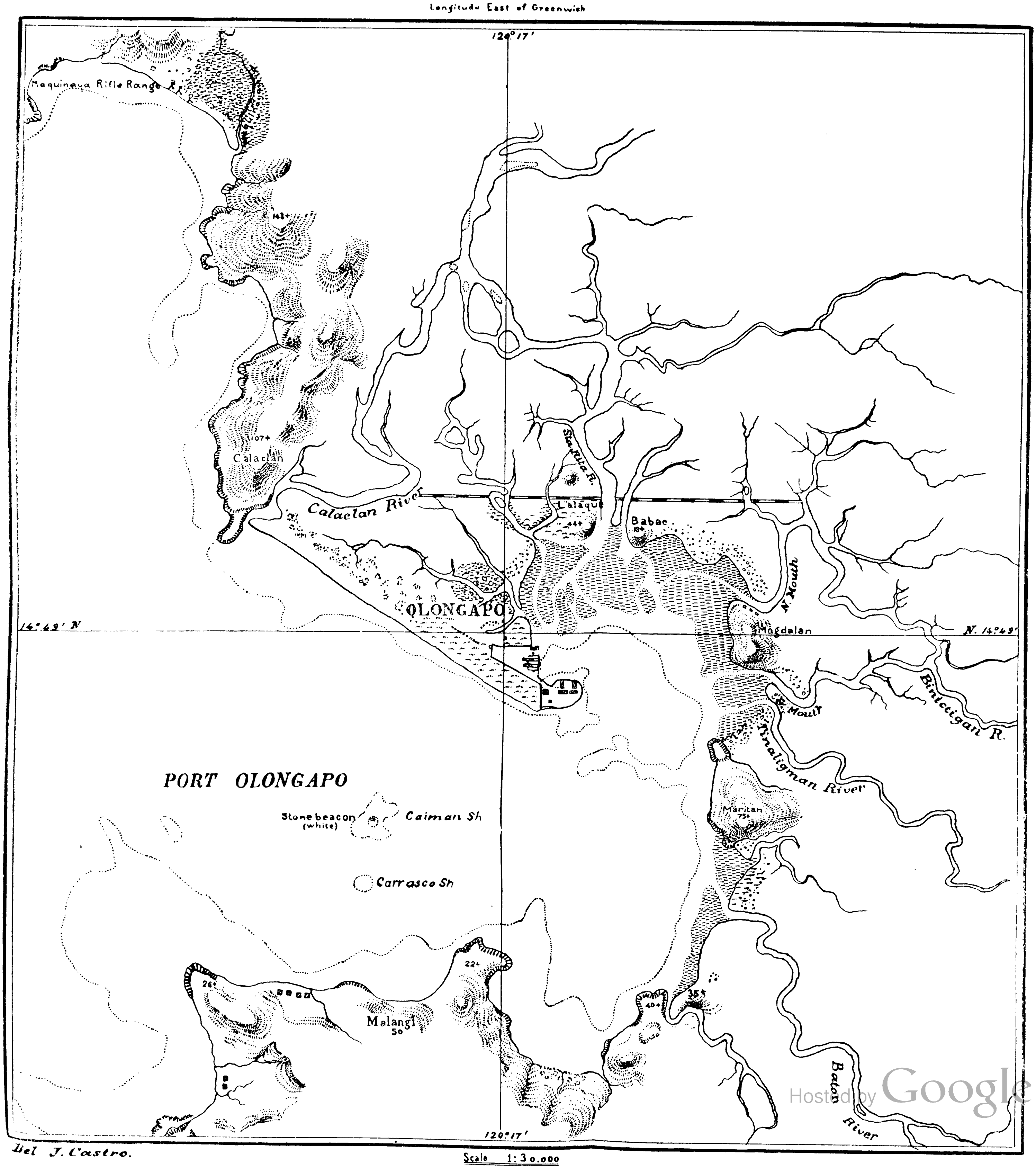
Clinical chart of J. C. B., upon whom experiments in inoculation with malarial parasites were performed.

PLACES OF *MYZOMYIA LUDLOWII THEOB.*,

APRIL-JUNE 1907.

BY CHARLES S. BANKS, ENTOMOLOGIST.

BUREAU OF SCIENCE



Del J. Castro.

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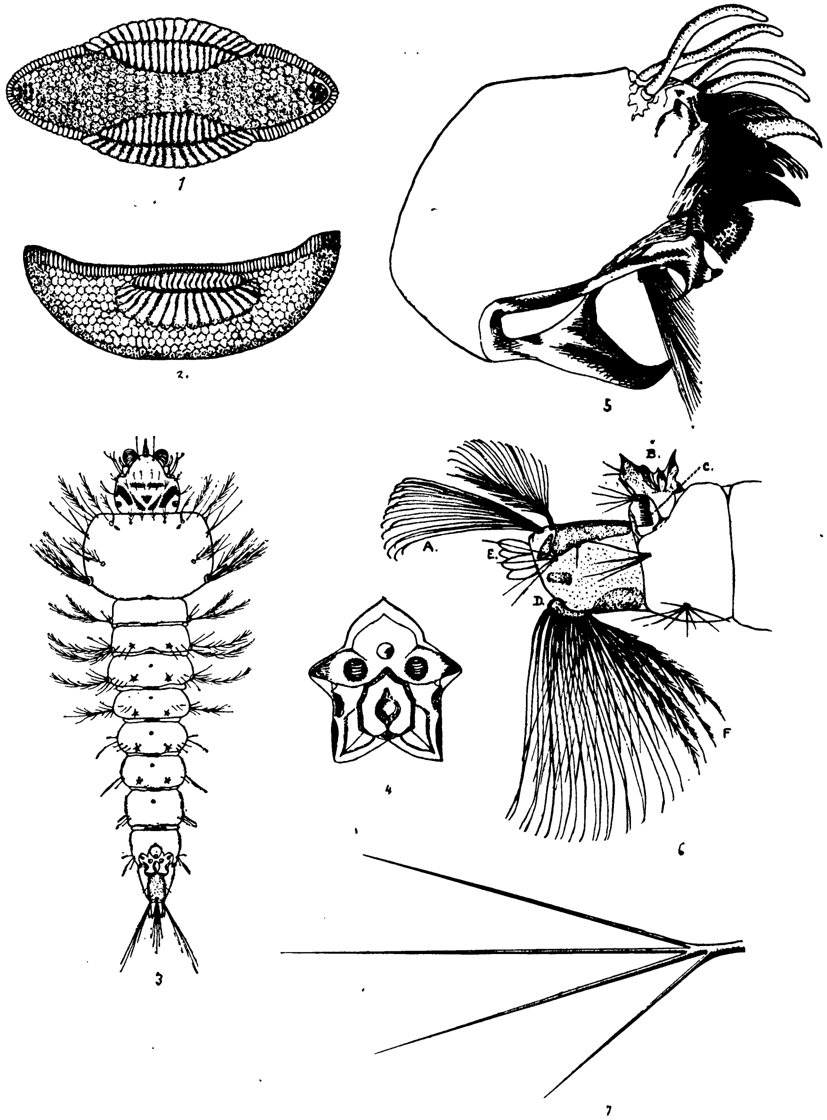


PLATE I.

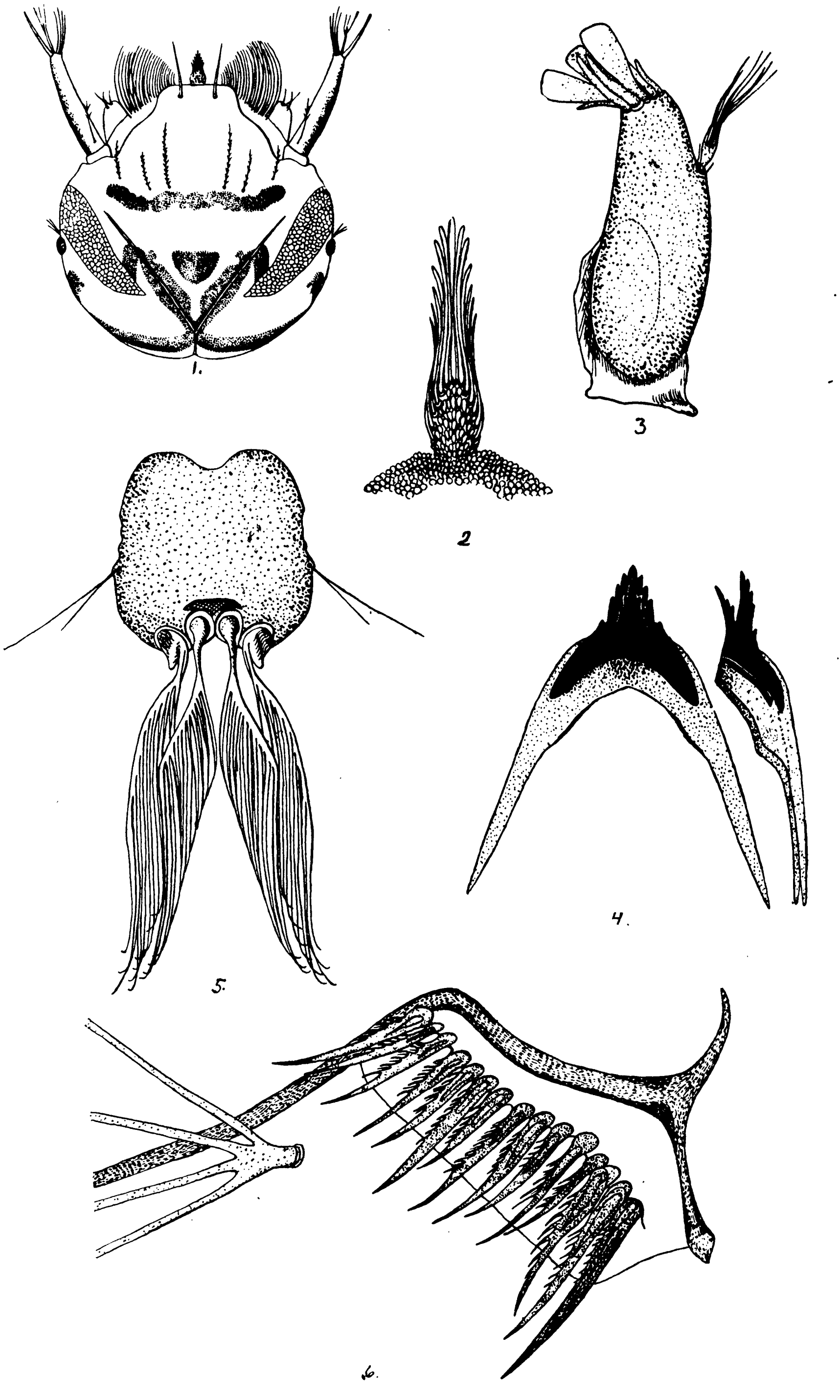
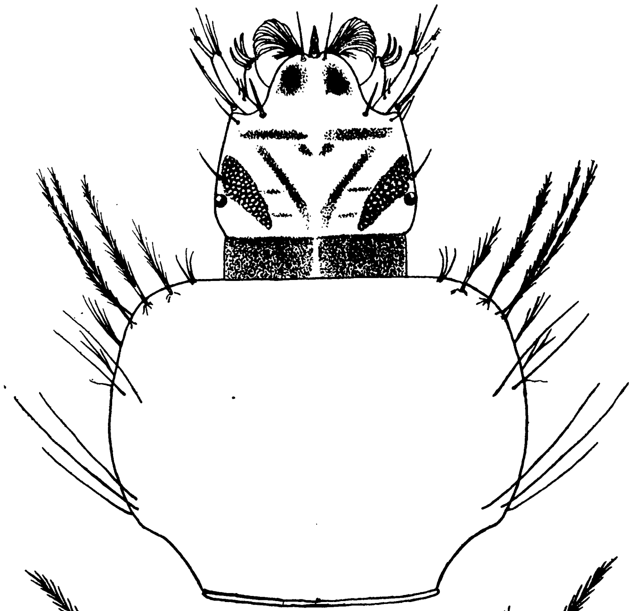


PLATE II.



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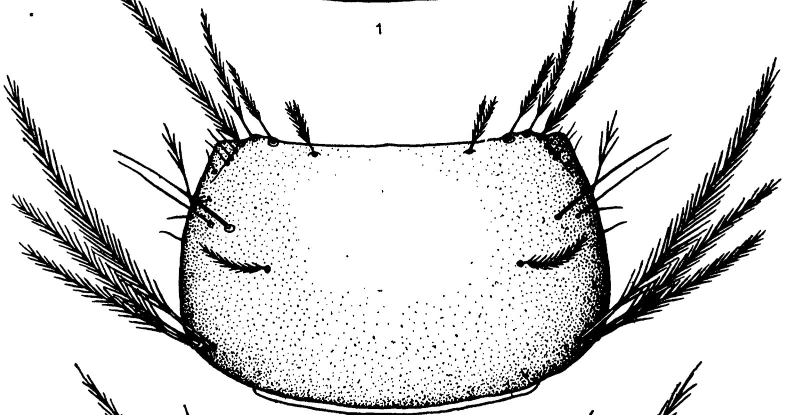

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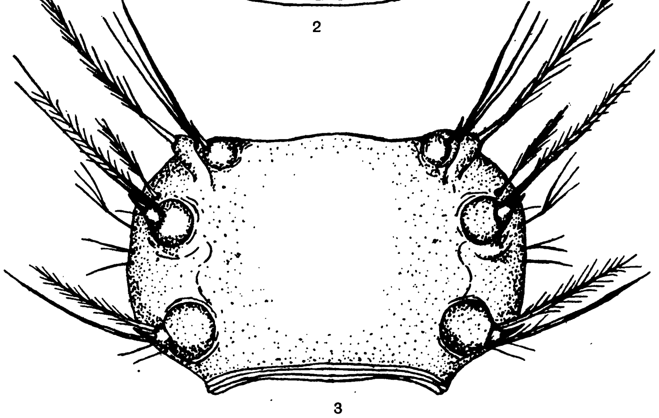

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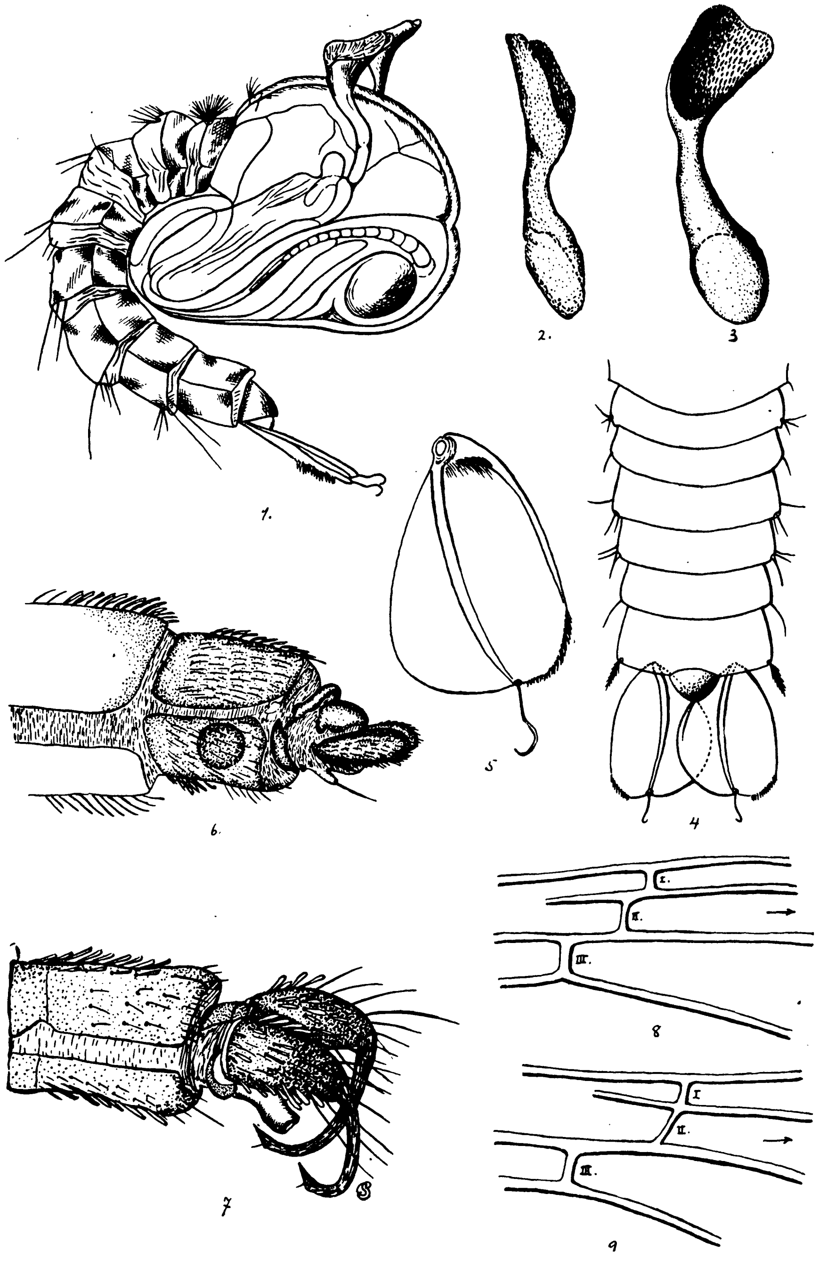
PLATE III.



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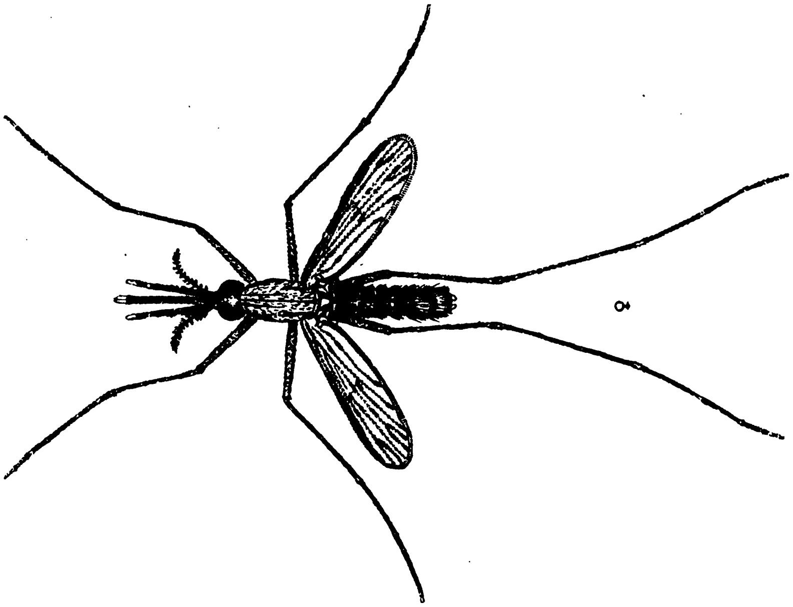


FIG. 1.

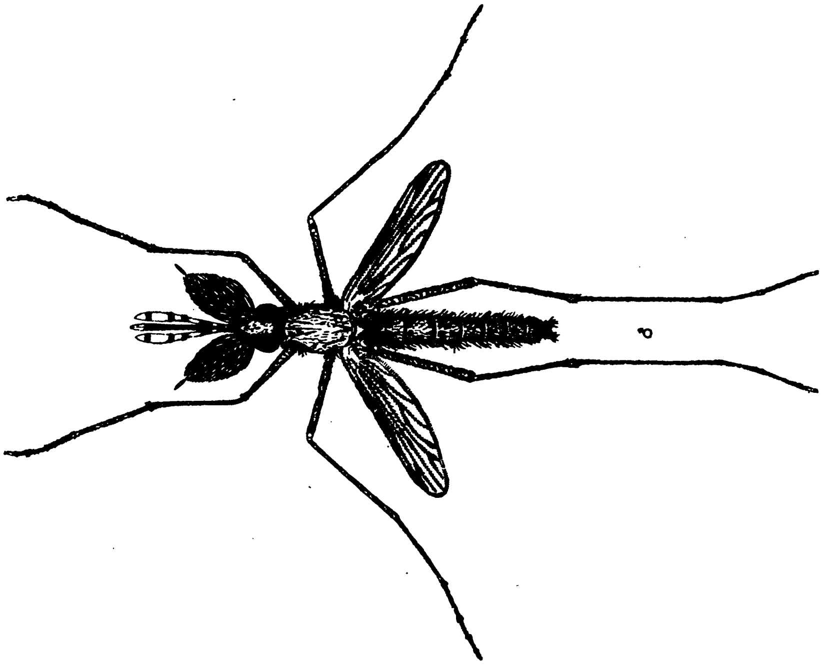


FIG. 2.

PLATE V.

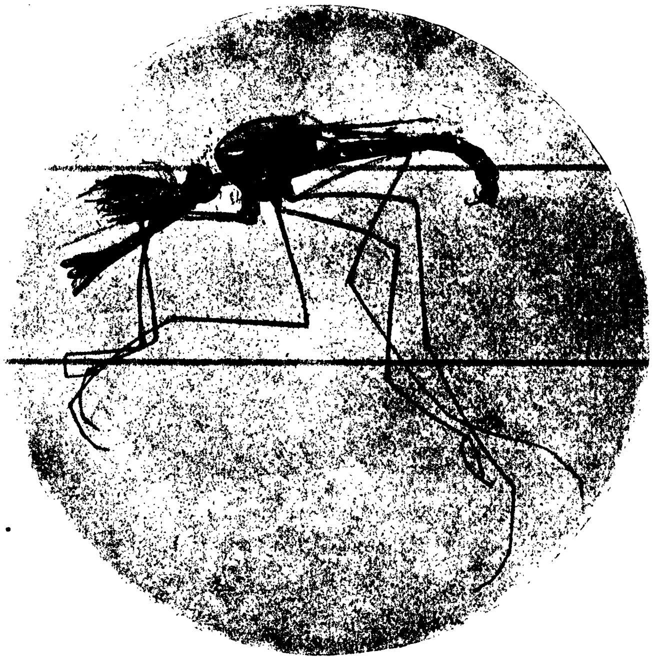


FIG. 1.

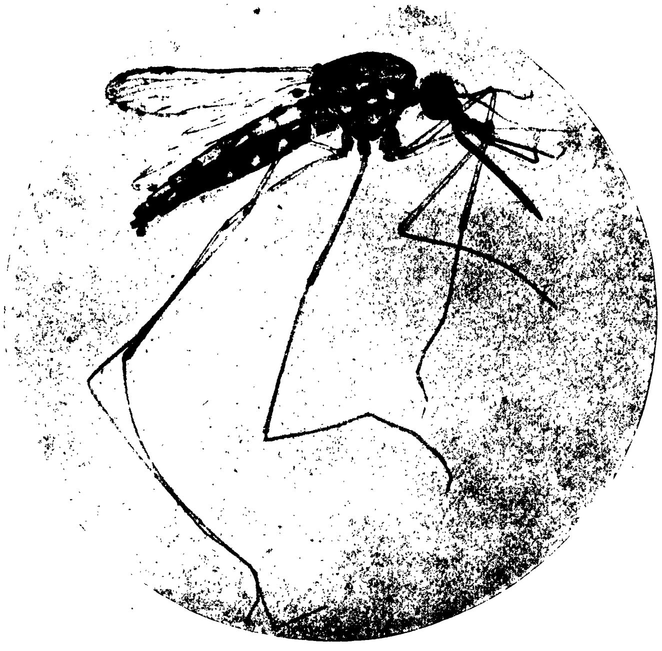


FIG. 2.

PLATE VI.

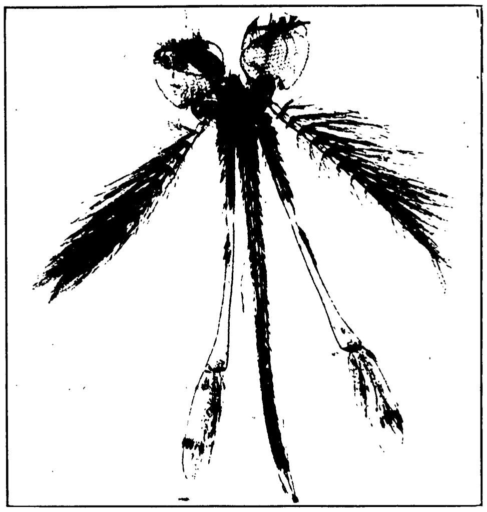


FIG. 1.

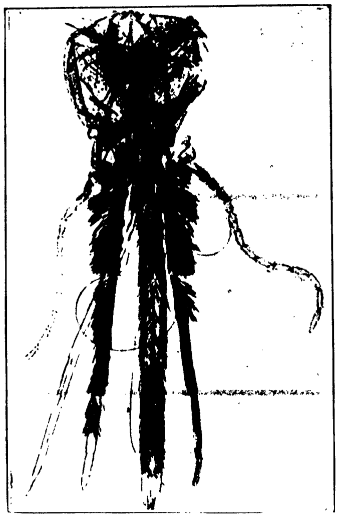


FIG. 2.

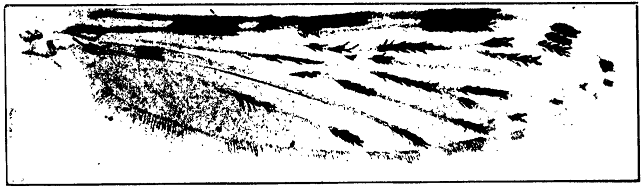


FIG. 3.

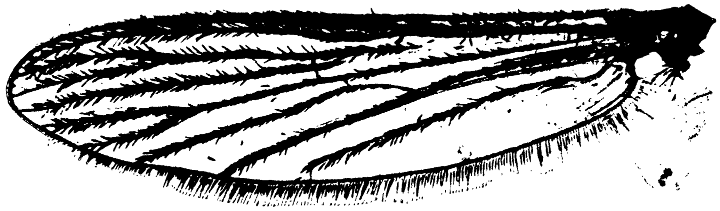


FIG. 4.

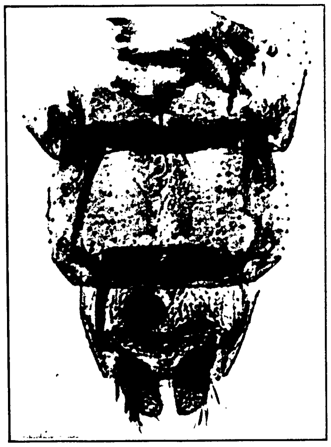


FIG. 5.

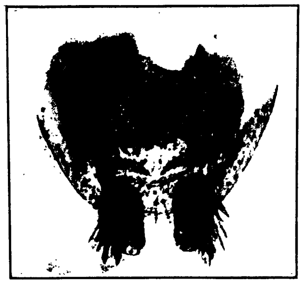


FIG. 6.

PLATE VII.

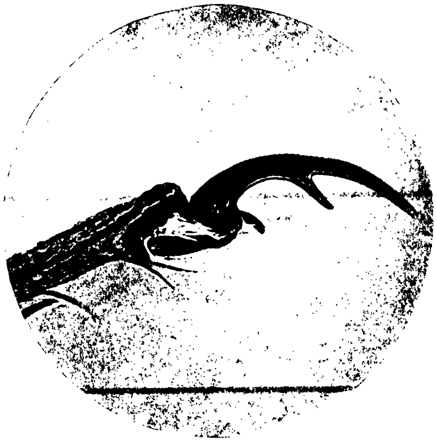


FIG. 1.

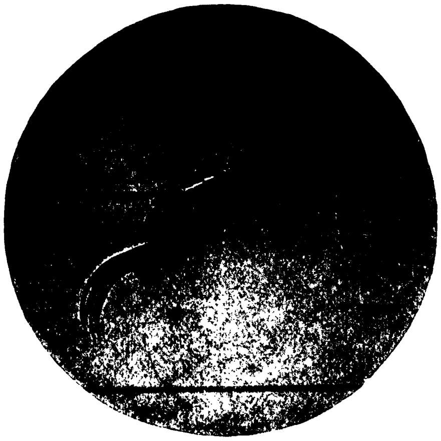


FIG. 2.

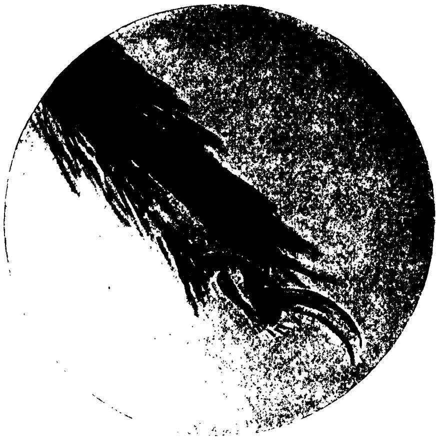


FIG 3

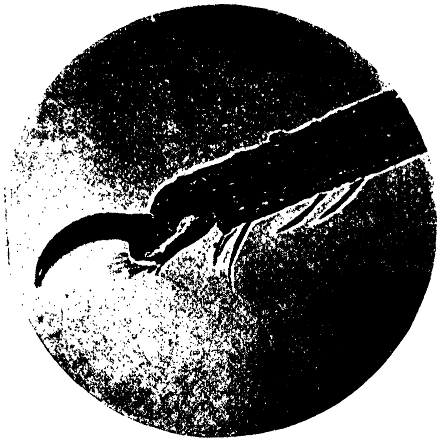


FIG. 4.

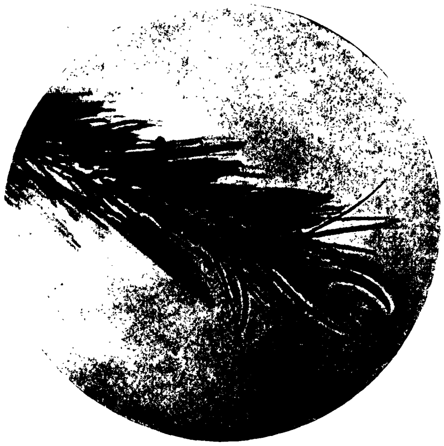


FIG. 5.

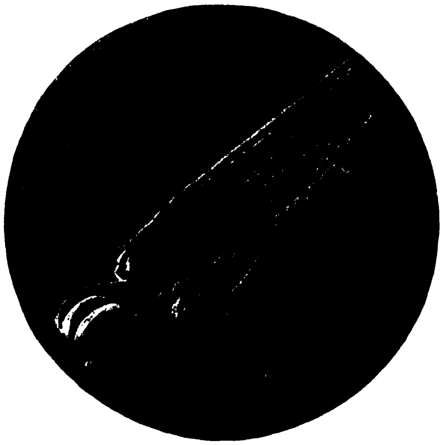


FIG. 6.

PLATE VIII.

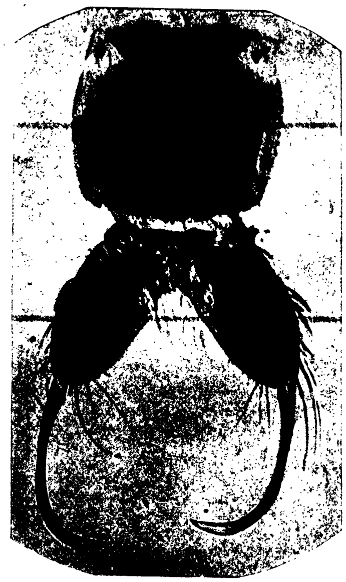


FIG. 1.

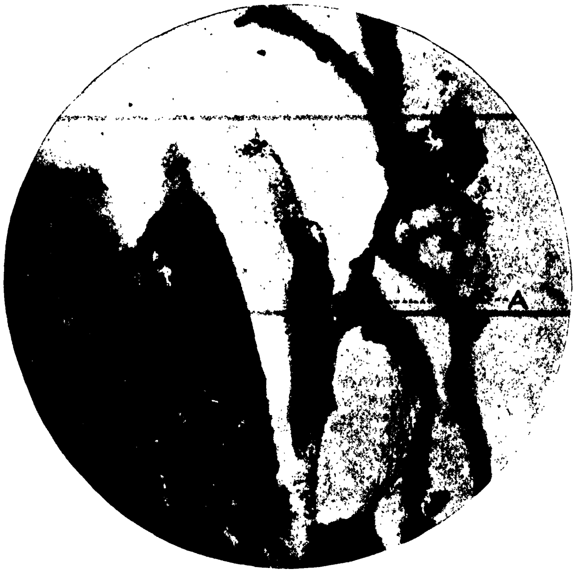


FIG. 2.

FIG. 3.

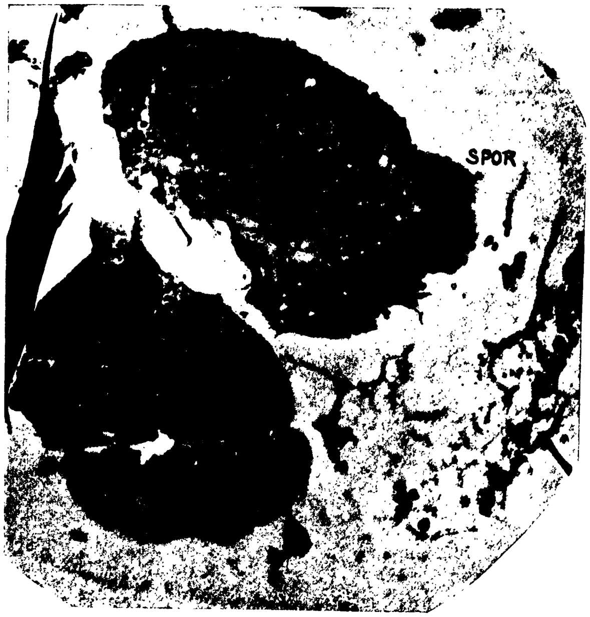


FIG. 4.

PLATE IX.

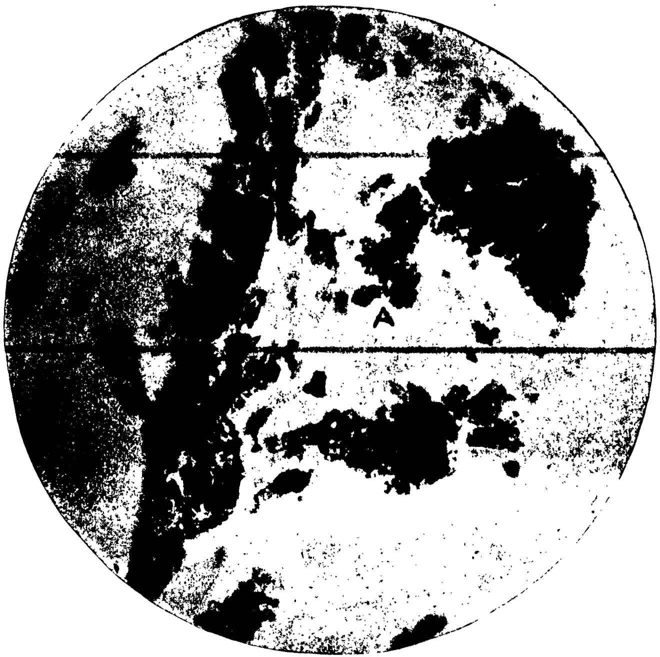
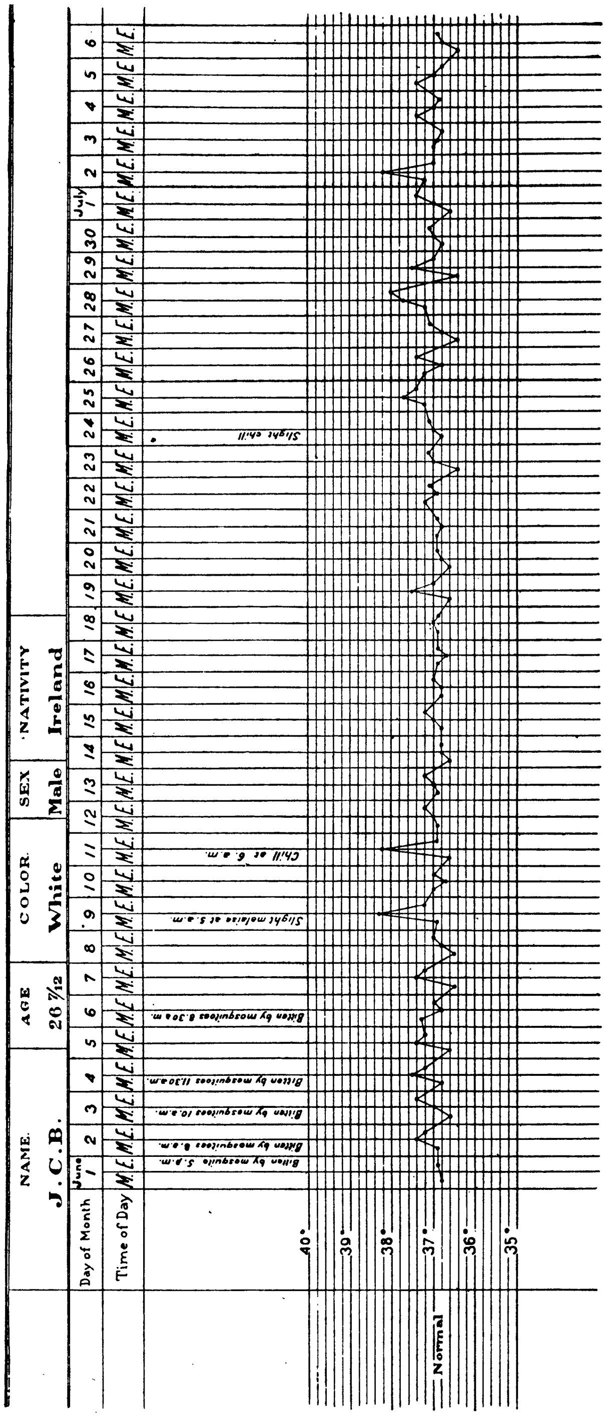


FIG. 1.

FIG. 2.

PLATE X.



Temperature Range 36 days-38.3°-36.4°-1.9°

PLATE XI.

A PRELIMINARY REPORT UPON THE SPECIFIC IDENTITY OF THE CESTODE PARASITES OF MAN IN THE PHILIPPINE ISLANDS WITH A DESCRIPTION OF A NEW SPECIES OF TÆNIA.

By PHILIP E. GARRISON.[1]

(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

INTRODUCTION.

The present paper embodies the results of careful determinative work upon such cestode material as has been collected for the Helminthological Collection of the Philippine Bureau of Science and is intended as the first of three or four papers, to appear as soon as the specimens in other groups can be properly worked over.

It is my purpose to endeavor to present in a systematic way some definite knowledge with regard to the specific identity of the more common animal parasites of man in the Philippine Islands. While the primary object of these publications is to clear the field for future work in the localities in question, rather than to present new helminthological findings, they naturally will include the description of forms coming to my attention in this preliminary work which seem to justify the establishment of new species or other systematic groups.

Our present knowledge of Philippine helminthology has been derived from a few determinations made by professional zoölogists upon material received from the Islands, from the reports of infections in Americans recently returned from a more or less prolonged residence in the Philippines, and from the work of resident medical scientists. While the information supplied by these sources has in many cases been lacking in the positive and precise identification of the parasites reported, it has established with reasonable certainty the presence in the Philippines of several forms already known in other parts of the world and has included the description of two new species of human entozoa, besides contributing considerable knowledge concerning the general prevalence of animal parasites in the Islands.

A summary of any results already published will be given in the consideration of each genus and species reported.

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[1] Assistant Surgeon, United States Navy; detailed medical zoölogist to the Bureau of Science, Manila, P. I.

MATERIAL.

The greater part of the material forming the basis of the present study has been collected from native prisoners in Bilibid Prison, a large proportion of whom were examined and treated in quarantine immediately upon their arrival from various provinces scattered throughout the Islands. Numerous other specimens have been received from patients in the private practice of physicians and in the hospitals of Manila.

The present reports deal simply with the identification of specimens collected and they do not undertake to consider the proportion of persons infected to those examined; therefore they can not properly be taken as an index to the frequency of infection in the population. However, considering that from 3,000 to 4,000 persons gathered from widely scattered localities have been examined for animal parasites at Bilibid during the current year, and that specimens have been received from various classes and conditions of social life, it would seem fairly probable that our Helminthological Collection at present contains representatives of most of the species of the parasites of man which are generally prevalent in the Islands, and furthermore that, with certain exceptions which will be noted in the text of this and the following papers, the collection may be accepted tentatively as a rough indication of the relative prevalence of the different species.[2]

CESTODA.

The relative infrequency of cestode infections compared to infection with the common nematodes, and on the other hand the greater familiarity with tapeworms and their relative innocuousness compared with trematodes, are probably responsible for the fact that, while valuable contributions have been made to the study of the *Nematoda, Trematoda,* and the *Protozoa* of the Philippines, the *Cestoda* have been almost entirely neglected.

So far as we have been able to discover, the following are the only published references to cestode infections in the Philippines:

Strong,[3] 1901, reporting the results of 1,793 stool examinations and 386 necropsies, states that only two adult *Tænia* had been found, but that both *T. saginata* and *T. solium* are present in the Islands, the former species being most frequently seen in American troops, the latter occasionally occurring among the natives.

In the annual reports of the Superintendent of Government Laboratories of the Philippine Bureau of Science for the years 1902 to 1905, inclusive,[4] in which Dr. Strong, Director of the Biological Laboratory, includes the results of over 6,000 microscopic examinations of faeces, only five cestode infections are reported, all with the genus *Tænia*, species not noted. Hallock,[5] at Fort Porter, Buffalo, N. Y., reported two infections with *Hymenolepis nana* in American soldiers recently returned from the Philippines. Stiles and Garrison,[6] at the Government Hospital for the Insane, District of Columbia, found no cestode infections in making microscopic examinations of the stools of 115 American soldiers who had returned from Philippine service. In 1906, Foster[7] reported two infections with *Hymenolepis nana* in American soldiers on duty near Manila.

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[2] I wish to acknowledge my indebtedness to Dr. Edwin C. Shattuck, resident physician of Bilibid Prison, for numerous specimens placed at my disposal and deposited in the Bureau of Science Helminthological Collection.

[3] *Circulars on Tropical Diseases*, Manila (1901), **1**, 15.

[4] *An. Rep. of the Superintendent of Government Laboratories,* Report of the Biological Laboratory, Manila (1902), 569; (1903), 413; (1904), 443; (1905), 357.

During the past nine months there have come to our attention about fifty intestinal infections with cestodes, representing three genera and five species.

TÆNIA.

Of the fifty cestode infections about forty were with worms of the genus *Tænia*. Specific diagnosis from the ova alone was not attempted. Thirty-one specimens of adult *Tænia* have been deposited in the collection.

The cases given in the annual reports of the Superintendent of Government Laboratories and the statement given by Strong in 1901, above noted, appear to be the only mention, at least within recent years, of the presence of this genus in the Philippines.

Tænia saginata Gœze, 1782. Twenty-six of the thirty-one *Tænia* specimens are *Tænia saginata,* twenty-two being from native Filipinos; fourteen possess the scolex and strobila entire, while twelve were determined from the strobila alone. Two other specimens which were received probably belong to this species, but owing to poor preservation the characters could not clearly be made out and the identity is considered doubtful. One worm in the recent state presented a fairly distinct post-suctorial ridge around the scolex, resembling that described for *Tænia hominis* Linstow, 1902. Further examination failed to reveal any other characters described for that species and seemed to establish the specimen's identity with *T. saginata.* In all cases presenting any peculiarities which might throw doubt upon the determination, the specimens were studied in stained sections in comparison with material of known identity.

Tænia philippina sp. nov. The establishment of this species is proposed upon a single specimen obtained in 1905 by Mr. Hare of the Bureau of Science, from a prisoner in Bilibid Prison and placed in the museum of the Bureau of Science without determination. For specific diagnosis and description of type specimen, see pp. 542–550, figs. 1–17.

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[5] *J. Am. Med. Ass.* (1904), **42**, 891.

[6] *Bull. Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv.*, Wash. (1906), No. **28**, 50, 51.

[7] *J. Am. Med. Ass.* (1906), **47**, 685, 686.

Tænia solium Linnaeus, 1758. Two specimens of the pork tapeworm were obtained from native prisoners in Bilibid. Although the scolex was absent from each specimen, the size of the strobilæ, the massive uterine branches, not more than fifteen in number, the "third ovarian lobe" cut off by the vagina and the absence of extreme lengthening of the terminal segments seemed to render the diagnosis reasonably certain.

The proportion of the number of specimens of *Tænia saginata* (26) to the number of specimens of *Tænia solium* (2) here reported would seem to indicate with fair probability that the former is the much more prevalent of the two species; however, further work is necessary before the relative frequency of infection by these two species can be established with even approximate accuracy.[8]

HYMENOLEPIS.

Five infections with this genus have come to our attention, one in a native child under the professional care of Surgeon D. N. Carpenter, United States Navy, at Cavite, and four in adult males at Bilibid Prison, one of whom was Chinese and the others native. Parasites were recovered from two of the latter cases after treatment. Specific diagnosis of the child's infection was made, it is believed, with fair certainty, from the ova.

Hymenolepis nana (Siebold, 1852) Blanchard, 1891. One undamaged specimen of this species was taken from one of the Bilibid prisoners. The size of the ova obtained from Dr. Carpenter's case (from 40 by 35 μ to 57 by 42 μ, outer shell) and the absence of a granular layer between the outer and inner shells seemed fairly safe evidence that the infection was with *H. nana* rather than with *H. diminuta*. A careful study of the ova was not made in two of the infections with *Hymenolepis* and worms could not be secured.

It must be borne in mind in forming a judgment from the cases here reported regarding the frequency of infection with the dwarf tapeworm in the Islands, that in other countries *Hymenolepis nana* has appeared to show a marked tendency to infect children more frequently than adults[9] and that our work has been almost exclusively with the latter.

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[8] The results of meat inspection in Manila by the Bureau of Agriculture show a much higher percentage of cysticercosis in hogs than in cattle and I was led to expect a correspondingly greater prevalence of the pork tapeworm. However, I have been informed by Dr. Moberly, chief veterinarian, that most of the animals slaughtered in Manila are imported, nearly all of the native hogs and cattle being killed in the provinces, where meat inspection has not yet been established. The results of inspection, therefore, are not indicative of the relative prevalence of *Cysticercus cellulosae* and of *C. bovis* in native animals.

[9] Cima, Francesco: *Pediatria Napoli* (1893), **1**, 39; *Ibid.* (1896), **4**, 303; Ransom, Brayton H.: *Bull. Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv.*, Wash. (1904), No. **18**, 61; Stiles, Ch. Wardell, and Garrison, Philip E.: *Ibid.* (1906), No. **24**, 24.

Hallock's two infections with *Hymenolepis nana* in American soldiers recently returned from service in the Philippines and the two cases of American soldiers serving in the Philippines reported by Foster (see page 539) are, so far as we are aware, the only published reports for this species in the Islands. Through very indirect channels we have heard of one unpublished case found by a medical officer of the United States Army in one of the southern islands. Further, Dr. W. E. Musgrave informs us that he has noticed the ova several times in making fœcal examinations of natives and Americans during the past few years.

Hymenolepis diminuta (Rudolphi, 1819) Blanchard, 1891. A stool from a Chinese prisoner at Bilibid was brought to us with the diagnosis of *Hymenolepis nana*. However, the ova were very large, and fortunately all doubts regarding the specific identity of the parasites were removed by finding in the specimen from ten to twelve adult worms, from 3 to 6 centimeters in length, with a rudimentary, unarmed rostellum. These, upon further study of stained specimens, gave all the characters of *Hymenolepis diminuta*.

Unless cases have been published too recently to have come to our attention in Manila, this is the thirteenth infection with *Hymenolepis diminuta* reported for man. Unfortunately, the prisoner was discharged the day the specimens were passed and no history was secured. Therefore, it must remain an open question whether the infection was imported or contracted in the Islands. There are two other specimens of this species in the Bureau of Science Collection which were obtained from rats in Manila.

DIBOTHRIOCEPHALUS.

No infections in man with parasites of this genus have been found. It is mentioned in this connection because of the strong probability that it is present among the natives and because of the published opinion of at least one prominent helminthologist[10] that it may be found to be an important parasite in the Philippines. This expressed probability is based upon the great importance of fish in the diet of the natives, upon the proximity of Japan, where *D. latus* has been found in man, and upon the further fact that the presence in the Islands of at least one representative of *Dibothriocephalus* has been established recently by finding in the intestine of a domestic cat a worm of this genus which closely resembles, if it is not identical with, *D. latus* in its specific characters.

DIPLOGONOPORUS.

Diplogonoporus grandis (Blanchard, 1894) Luche, 1899, the double-pored, Japanese tapeworm, has also been mentioned as a possible Philippine parasite upon much the same grounds as *Dibothriocephalus*. Up to the present time it has not been observed.

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[10] Stiles, Ch. Wardell: *Ibid.* (1906), No. **25**, 9.

SOMATIC CESTODE INFECTIONS.

No human infections with larval cestodes have been found. Strong,[11] reports two human infections with larval *Echinococcus* and upon inquiry we have learned of two or three cases of cysticercosis and of at least one other of echinococcus infection occurring in Manila within a few years. These verbal reports are all more or less indefinite and no specimens appear to have been preserved.

Two specimens of pork infected with *Cysticercus cellulosæ* have been received and this parasite appears to be rather common among the native hogs. The collection of the Bureau of Science also contains two specimens of adult *Echinococcus* [12] obtained from native dogs.

Tænia philippina sp. nov.

SPECIFIC DIAGNOSIS.—*Tænia* (*Tæniarhynchus*): Strobila 80 to 100 centimeters in length; 1 centimeter in maximum breadth; composed of about 800 proglottides. Head cuboid, 1 to 1.5 millimeters in diameter: suckers 0.35 by 0.40 millimeter; their lumena cylindrical to conical, directed forward and outward, 0.22 to 0.26 millimeter deep by 0.19 to 0.21 millimeter broad at their bases. Neck segmented, its minimum transverse diameter, 0.70 millimeter, at about the 75th segment and about 3 millimeters from posterior border of head. Sexual maturity first present at from 15 to 18 centimeters from anterior extremity and in about the 470th segment. Sexually mature proglottides 4 to 5 millimeters transversely by 0.8 to 1 millimeter longitudinally by from 1 to 1.3 millimeters dorso-ventrally; genital pore very slightly posterior to middle of lateral margin; cloaca 0.32 millimeter deep; cirrus distinct, 0.1 millimeter long by 0.03 millimeter broad; cirrus pouch 0.38 millimeter long by 0.10 millimeter broad; vas deferens forms loop within cirrus pouch, is without vesicula seminalis, and ends in a dilated extremity before reaching median line; testicles 0.13 to 0.16 millimeter long by 0.06 to 0.08 millimeter in their transverse diameters; vagina without setæ and forms two spiral coils between cloaca and excretory canal; vitellogen gland greatly elongated transversely, lies close against transverse canal, filling posterior one-tenth of medullary space in median line, its extremities attenuated and extending laterally two-thirds the distance from median line to excretory canals; ovaries composed of two unequal, transversely elongated lobes which lie close against the vitellogen gland posteriorly and extend laterally through three-fourths the distance from median line to excretory canals; the smaller lobe, proximal to pore of segment, is bounded internally by the median line and anteriorly by the vagina; the larger, distal lobe extends anteriorly to transverse plane passed through genital pore, its anterior third projecting across the median line, overlapping anteriorly the median extremity of the opposite lobe and filling the space between the two limbs of the posterior V-shaped portion of the uterus; the uterine stem presents a V-shaped course in the posterior half of the segment with its apex to the pore side of segment, its posterior extremity lying

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[11] *Circulars on Tropical Diseases* (1901), **1**, 22.

[12] *Echinococcus granulosus* (Batsch, 1786) Rudolphi, 1805 = *Tænia echinococcus* Siebold, 1853. (See Stiles, Ch. Wardell, *Bull. Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv.*, Wash. (1906), No. **25**, 75 et seq.

in the median line between the ovarian lobes, its anterior limb turning directly cephalad in the median line at about the center of the segment and presenting, in the anterior half of the segment, a spiral formation consisting of from four to six distinct, closely wound coils. In the gravid segment, the uterus presents an exceedingly compact formation consisting of a median stem and numerous long, slender, dichotomous branches, separated by thin partitions and frequently overlapping one another dorso-ventrally. Terminal segments equilateral or slightly longer than broad, measuring from 6 to 8 millimeters in longitudinal and transverse diameters and 3 millimeters dorso-ventrally. Embryophore oval, 35 to 41 μ long by 26 to 35 μ broad.

CYSTICERCUS unknown.

TYPE HOST.—*Homo sapiens.*

TYPE LOCALITY.—MANILA, P. I.

TYPE SPECIMEN.—No. 140, Helminthological Collection, Bureau of Science, Manila, P. I.

DESCRIPTION OF TYPE SPECIMEN.

Length 82.8 centimeters: transverse and dorso-ventral diameters respectively; of head, 1.5 millimeters by 0.75 millimeter; of neck, 0.72 by 0.24 millimeter; of mature proglottides, 10 by 1.3 millimeters; of terminal proglottides, 6 to 8 by 3 millimeters. Indentations of lateral margins suggesting segmentation are present close behind suckers; distinct segmentation appears 1 millimeter behind suckers; greatest constriction of neck, 3.5 millimeters behind suckers. (Figs. 2 to 4.) Transverse diameters of segments greater than longitudinal diameters except in terminal proglottides which are equilateral or slightly longer than broad. (Figs. 1, 18.) Genital pores prominent, and slightly behind middle of lateral margins of segments. (Figs. 1, 16 to 18.) Surfaces and margins of segments extend posteriorly in an elongated, cuff-like projection overlapping the succeeding segment and are indented by transverse infoldings of the cuticle, which appear to be formed in part, and perhaps wholly, by longitudinal contraction of the specimen. (Figs. 1, 5 to 7.) Calcareous corpuscles absent.

Head.—Transverse diameter, 1.5 millimeters; dorso-ventral diameter 0.75 millimeter, the lesser, dorso-ventral diameter appearing to be due to flattening by cover-glass pressure. Other dimensions as follows: From tip of head to plane passed through anterior borders of suckers, 0.15 millimeter, to posterior border of suckers, 0.64 millimeter; approximate longitudinal diameter of head, 1 millimeter. Rostellum (fig. 3) without hooks and retracted completely within head, its musculature globular, 0.16 millimeter in longitudinal by 0.112 millimeter in transverse diameter; slight depression at tip marks opening of lumen of minute apical sucker. Suckers (figs. 2 and 3) well embedded in musculature of head at its dorso- and ventro-lateral borders with the longitudinal axis of their lumena directed forward and outward; transverse diameter, 0.34 to 0.37 millimeter; longitudinal diameter, 0.37 to 0.4 millimeter; lumena cylindrical to conical, 0.224 to 0.260 millimeter deep by 0.19 to 0.21 millimeter broad at their bases.

Nervous system.—Situated 0.8 millimeter from anterior extremity of the head and 0.15 millimeter behind plane passed through posterior border of suckers, a large cavity (0.25 by 0.20 millimeter) incloses what appears to be a contracted, globular nerve ganglion (0.16 by 0.11 millimeter). (Fig. 3.) The nerve trunks proceed posteriorly in a straight course, at first dorso-lateral and later directly lateral of ventral excretory canals; concomitant dorsal and ventral fasciculi not apparent; lateral nerve trunks rather crescentic on cross section and measure 0.025 millimeter transversely by 0.150 dorso-ventrally in mature segments.[13]

Excretory system.—The ventral excretory canals pass in a straight course from the head through the lateral borders of the strobila, at first ventrad to and later directly in the median saggital plane. They attain their maximum diameter in the first gravid proglottides (0.35 to 0.40 millimeter in median transverse plane) and present a wide dilation (0.45 to 0.65 millimeter longitudinally by 0.80 to 0.90 millimeter transversely and dorso-ventrally) at each segmental junction. (Figs. 7, 8, 16, 17.) The transverse canals decrease in size as they pass inward from the dilated portions of the ventral canals, are oval or diamond shaped on cross section (figs. 5, 10) and are situated at the extreme posterior borders of the proximal segments, it being impossible in either young, mature, or gravid proglottides, to detect any extension of the proximal segment posterior to the canal. (Figs. 5, 8, 13, 17.)

The dorsal excretory canals can be traced as loosely wound, spiral tubules, 18 to 20 μ in diameter, as far as the first sexually mature proglottides. The coils of the spiral extend through a space of from 0.15 to 0.25 millimeter transversely and dorso-ventrally and number five to six complete turns in each segment. (Figs. 5, 7.)

Genital organs.—The primordium of the vas deferens and vagina appears in about the 150th segment, which measures 0.72 millimeter transversely by 0.24 millimeter longitudinally, and is situated 12 millimeters from anterior extremity. At about the 300th segment (1.75 millimeter by 0.45 millimeter, 2 centimeters from anterior extremity) the uterine stem is present as a distinct tract beginning posteriorly in the bulbous mass at the median extremity of the vagina, and passing diagonally anterolaterally toward the pore side of the segment until it reaches the angle between the vagina and vas deferens, which are now separated throughout the inner half of their course; thence turning at an acute angle, it takes an antero-median course to the median line, and along the median line to the anterior border of the segment. (Figs. 6, 7.) In the 400th segment (3 millimeters by 0.64 millimeter, 6 centimeters from anterior extremity) the female organs have became differentiated into the ovarian lobes and vitellogen gland, the testicles are appearing in lateral fields of medullary layer, the cloaca is forming at the lateral margin, the V-shaped course of the uterine stem in the posterior half of the segment is more sharply defined and its anterior prolongation in the median line is coiled in one or two loose spiral turns. The 475th proglottid is sexually mature. (Figs. 8, 10.)

Sexually mature proglottid.—Measurements: 5 millimeters transversely by 1 millimeter longitudinally by 1.35 millimeters dorso-ventrally, situated 15.5 centimeters from anterior extremity of specimen. Genital pore slightly posterior to middle of lateral margin (fig. 8); cloaca 0.32 millimeter deep (figs. 8, 12, 15); cirrus 0.1 millimeter long by 0.03 millimeter broad (fig. 15); cirrus pouch 0.35 millimeter long by 0.1 millimeter broad (fig. 15); vas deferens makes distinct

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[13] In the absence of specific staining for the nerve tissues, the nature of the globular structure in the posterior part of the head can not definitely be determined. The interpretation here made is based upon the marked similarity in appearance and structure in cross section between the lateral nerve trunks and the body in question when stained with carmine, upon the faint tracings of what appear to be nerve trunks arising from it, upon its position, and upon the elimination of other possible interpretations. The presence of a large, globular, well-defined ganglion as the center of the nervous system instead of the usual commissural arrangement of fibers would be an important character, but in view of the uncertainty of interpretation, it is not included in the diagnosis.

loop within cirrus pouch (fig. 15), is extremely convoluted after passing ventral canal, and ends shortly before reaching median line in distinct dilation (0.075 millimeter broad) which receives numerous vasa efferentia; testicles (figs. 8, 9, 13) measure 0.13 to 0.16 millimeter in length (dorso-ventral diameter of segment) by 0.06 to 0.08 millimeter in their transverse diameters.

Vagina makes two spiral coils before reaching excretory canal (figs. 11, 12) and after passing dorsad of the canal turns sharply ventrad, sweeps posteriorly, and near the median line it dilates to about twice its former diameter to form a muscular receptaculum seminis (0.040 millimeter in diameter) which abruptly enters a large globular chamber (0.16 millimeter in diameter) situated ventrally in the median line between the median extremities of the ovaries and the vitellogen gland and containing immature ova. (Figs. 7, 8, 13, 14.) From the posterior border of the chamber a tubule, 0.05 millimeter in diameter, passes dorsally in a convoluted course, taking up the vitellogen and shell gland ducts, and empties in the dilated posterior extremity of the uterus.

The vitellogen gland (figs. 8, 10, 13) lies along the extreme posterior border, close against the transverse canal, extending laterally two-thirds the distance from the median line to the ventral canals (1.2 millimeters). On longitudinal section in the median line it measures 0.3 millimeter dorso-ventrally by 0.01 millimeter longitudinally, filling the posterior one-tenth of the medullary space. Its lateral extremities are attenuated and lie entirely ventrad to the median saggital plane.

The ovary (figs. 8, 10, 13) is composed of two unequal, transversely elongated lobes with their posterior and ventral surfaces lying close against the vitellogen gland and the ventral boundary of the medullary layer respectively. Each lobe extends laterally three-fourths the distance from the median line to the ventral canals and is thickest in the posterior half of its median third where it completely fills the medullary space dorso-ventrally. The dorsal surfaces of the lobes are convex, approaching the ventral boundary of the medullary space at their median, anterior, and lateral margins.

The lesser lobe, proximal to the pore side of segment, is bounded anteriorly by the course of the vagina, except in its median third where, as it approaches the median line, a few filaments may pass ventrad to the vagina and reach a more anterior position.

The greater lobe, distad to the pore, extends anteriorly to or slightly beyond a transverse plane passed through genital pore; its anterior third is extended across the median line, slightly overlapping anteriorly the inner extremity of the opposite lobe.

The uterus (figs. 8 to 10) begins posteriorly as a pyriform dilation, 0.45 millimeter in diameter, situated obliquely between, but slightly dorsad to, the median borders of the ovarian lobes; diminishing in size the uterine stem passes obliquely antero-laterally toward the pore side of the segment until it reaches a point posterior to the median extremity of the vas deferens, when it turns inwardly at a sharp angle to its former course and, passing around the extended extremity of the greater ovarian lobe, continues obliquely forward and inward to the median line, which it reaches at about the center of the longitudinal axis of the segment; it then turns anteriorly and, in a spiral course of from four to six closely wound turns, reaches the anterior segmental border. In the spiral anterior limb the diameter of the uterine tube is about 0.035 millimeter, while the spiral fills a space of from 0.080 to 0.130 millimeter, the spiral coils becoming wider as they approach the anterior border of the segment.

Further development of the uterus (figs. 13, 16 to 18).—In the 500th segment (fig. 13) the dilated posterior extremity of the uterine tube has enlarged to a chamber measuring 0.32 millimeter dorso-ventrally and antero-posteriorly, by

0.8 millimeter transversely, which nearly fills the posterior portion of the medullary space in the median one-sixth of the segment. This joins a second dilation of about equal dimensions situated at the apex of the V-shaped portion of the uterine tube. In the anterior half of the segment the original four to six spiral turns are still distinct. The dilated, outer walls of the coils fill the dorso-ventral diameter of the medullary space, extend 0.4 millimeter on each side the median line, and project prominently forward into the transverse excretory canal. A number of shallow diverticula have grown out from the lateral walls of both the two posterior cavities and of the anterior spiral.

In segments 550 to 560 (fig. 16) from five to seven diverticula or branches have grown out from the lateral walls of the two posterior uterine dilations, extending laterally 1.17 millimeters or four-fifths the distance from the median line to the ventral canals. In the anterior half of the segments the original four to six coils of the spiral formation are distinguishable, and extend laterally 1.30 millimeters from the median line, or about two-thirds the distance to the lateral canals. The anterior extremity of the spiral has expanded laterally into a cavity which encroaches upon the lumen of the transverse canal. As they extend toward the lateral borders, the anterior coils and the posterior diverticula apparently by reason of mutual pressure and unequal growth, have more or less lost the regularity of their earlier structure, some pressing toward the dorsal and others toward the ventral surface and some are inclosed at their lateral extremities by two adjacent and more laterally extended outgrowths. As a result of this irregular development, saggital and longitudinal sections in different planes present widely differing pictures, resembling in some cases the uterine branches extending laterally from a median stem as in other species of *Tœnia* and in others giving the appearance of the egg-sack formation found in other genera.

As a whole, the structure is remarkably compact, the ovic chambers being closely pressed together and the partitions between them extremely thin.

In the terminal proglottides (figs. 17, 18) the uterus fills the entire medullary space, encroaches upon the transverse and longitudinal canals, and short diverticula even press outward the muscular tissue of the cortex. A few of the outgrowths reach the lateral canals without division and in the same plane. Usually, their lateral extremities divide into two or three secondary branches, which may again divide, the divisions taking place in any plane, but usually antero-posteriorly. In a median saggital section it is sometimes possible to follow a single branch from the median line, or even slightly across it, to the lateral canal, but in most cases it passes dorsad or ventrad and its further lateral course can be followed only in sections in other planes. Occasionally, a branch ends considerably short of the lateral canals, other branches closing in around its lateral extremity.

Ova.—An outer shell could not be distinguished in either young or matured ova. The embryophore is oval, with a thick, radially striated shell. Measurement of fifty embryophores gave the following results in microns:

32.5×30	37 ×30	35 ×31.5	38.9×33.3	37 ×33.3
37 ×31.5	40 ×31.5	36 ×29.6	37 ×33.3	37.5×33
37.5×29.6	38 ×31.5	38 ×28	40.7×33.3	36 ×28
36 ×27	37 ×33.3	37.5×26	40.7×33.3	37 ×31.5
38 ×28.5	40.7×33.3	38 ×33	37 ×28	37 ×34
39 ×29	40 ×33	40.7×33.3	36.5×29.6	37 ×32
37 ×29.6	38.9×35	37 ×30	37 ×30	38.8×33.3
40.7×32	35 ×29.6	37 ×33	40 ×29.6	40.7×33.3
37.5×33.5	34 ×29.6	40.7×33.3	38.5×29.6	37 ×29.6
37 ×29.6	38 ×33.3	37 ×30	37 ×27	39 ×33

Maximum dimensions, 40.7×35 μ; minimum, 35×26 μ; average, 37.8×31.1 μ.

DISCUSSION.

The type specimen, although preserved for about two years in formalin, was in good condition, except that it was considerably, although apparently uniformly, contracted. In the description of the specimen, actual measurements are given, but in stating dimensions in the specific diagnosis I have endeavored to make a safe allowance for this contracted condition of the specimen and for individual differences which other specimens may present. While the examination of further material may lead to changes in the diagnosis of the species, either by the elimination of some characters now included or by the addition of new ones, it is believed that the principal morphological features described, namely, the transversely elongated ovarian lobes and vitellogen gland, the V and spiral formation of the uterine stem, the compact structure of the gravid uterus, the loop of the vas deferens, the length of the strobila, and the relative length and breadth of the individual segments, form a combination of characters which could neither be accounted for by peculiarities in the living individual nor by its post-mortem distortion.

We have, upon the presence of the rudimentary unarmed rostellum, placed the species tentatively in the subgenus *Tæniarhynchus* Weinland, 1853, pending the examination of the material by a systematic zoölogist.

ILLUSTRATIONS.

[Fig. 1 is from unstained material. All other material was stained with acid carmine, picric acid being used in some cases for counterstaining. Sections vary from 25 to 250 μ in thickness. The magnification was governed with the view of bringing out as well as possible the desired structures, rather than by the consideration of uniformity.]

PLATE I.

FIG. 1. Portion of strobila. Natural size.

2. Section of head through two suckers and border of neural cavity. 1×75.

3. Section of head in different plane, showing (?) neural cavity and ganglion, retracted rostellum and traces of excretory canals. 1×75.

4. Longitudinal section through neck, showing beginning of segmentation. 1×30.

PLATE II.

FIG. 5. Longitudinal sections of segments 372, 373 just internal to ventral excretory canal, showing spiral course of dorsal canal. 1×80.

6. Segments 375 to 397, showing the early appearance of the uterus and genital pore. 1×10.

7. Saggital sections of segments 398 to 403, showing in different planes the early appearance of genital organs. 1×12.

PLATE III.

FIG. 8. Saggital sections of segments 451 to 455, showing sexual organs about mature. 1×12.

9. The same, highly magnified to show the form of uterus in a single segment. 1×98.

10. Transverse section of segment, showing the coils of vagina between cloaca and ventral canal. 1×80.

11. Saggital section of a younger segment showing the same. 1×82.

12. Transverse section of segment showing genital pore, cirrus, and loop of vas deferens in cirrus pouch. 1×90.

PLATE IV.

FIG. 13. Saggital sections of segments 493 to 495 in slightly different planes, showing posterior dilations of the uterus and its anterior coils which have nearly reached their maximum expansion before dividing. The heavily stained vitellogen gland extending along the transverse canal, and the more lightly stained ovarian lobes lying closely anterior to it are at their greatest development. 1×20.

14. Saggital section of segment, in a ventral plane, highly magnified to show inner length of vagina as it enters globular chamber from which the ovospermatic duct proceeds posteriorly toward the vitellogen gland and turns dorsally toward the shell gland which lies in a deeper plane. 1×40.

15. Longitudinal median section of segment 475, showing below, the vitellogen gland resting on the transverse canal; above and to the left the shell gland, above which the posterior extremity of uterus is faintly visible; more anteriorly the inner extremity of the greater ovarian lobe appears as it crosses the median line; above it are seen the well-expanded coils of the uterus. 1×60.

PLATE V.

FIG. 16. Saggital section of segments 555 to 556, showing further development of the uterus; numerous diverticula have spread laterally, in different planes from the outer walls of the uterine dilations and spiral coils. 1×10.

17. Saggital sections of segments 647 to 648 showing the irregular, compact structure of the uterus in gravid segments. 1×12.

18. Segment slightly compressed, showing gravid uterus. 1×10.

FIG. 1.

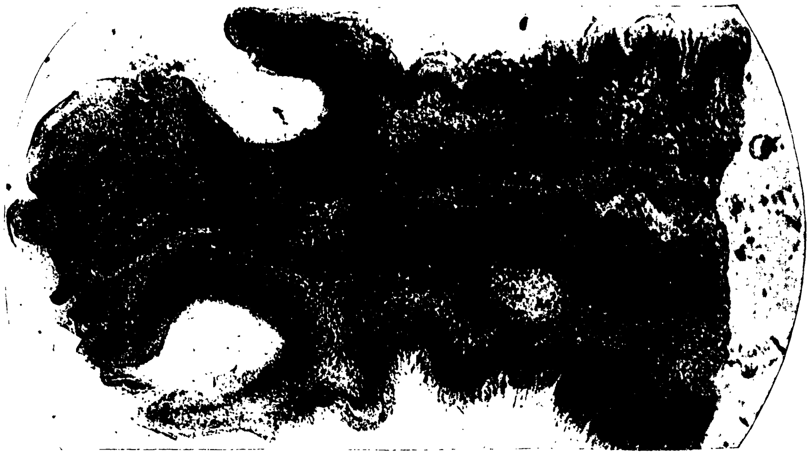

FIG. 2.

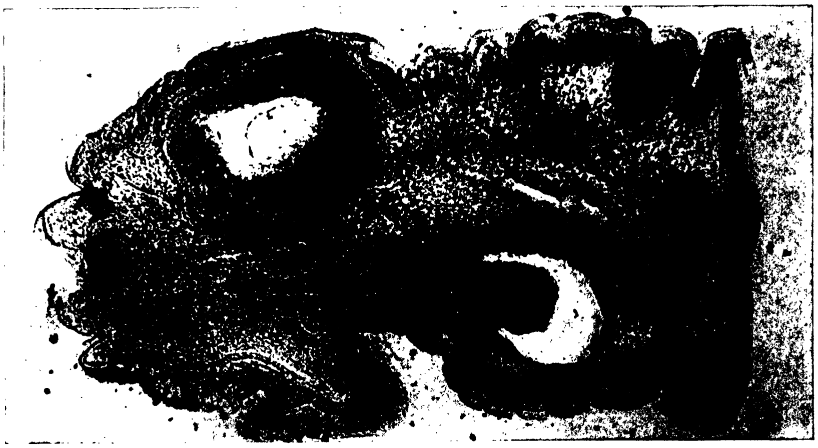

FIG. 3.

FIG. 4.

PLATE I.

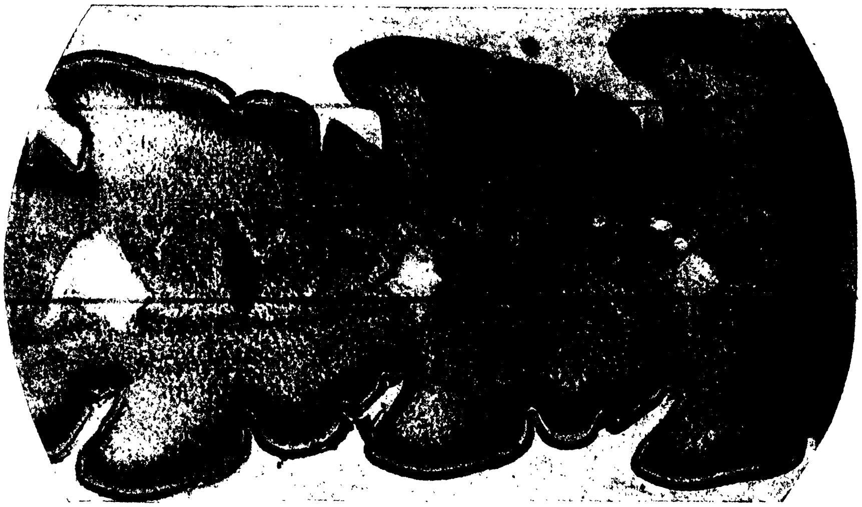

FIG. 5.

FIG. 6.

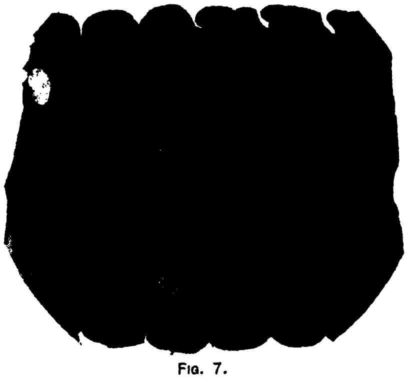

FIG. 7.

PLATE II.

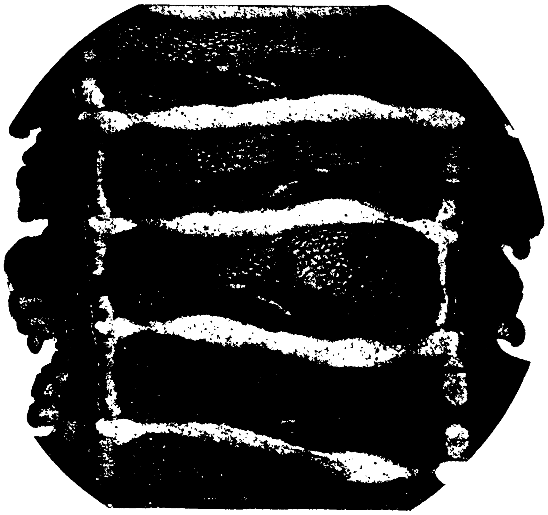

FIG. 8.

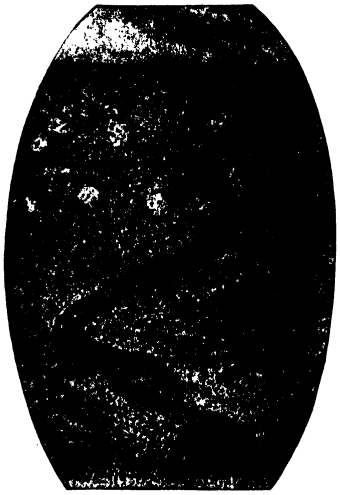

FIG. 9.

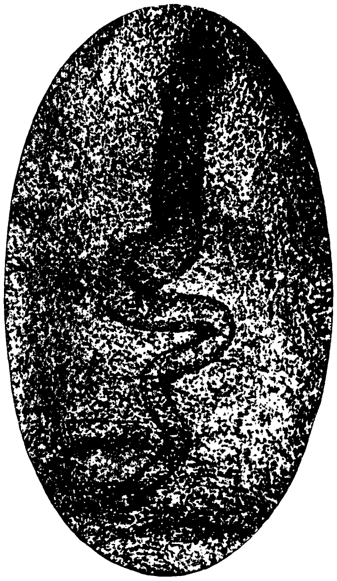

FIG. 10.

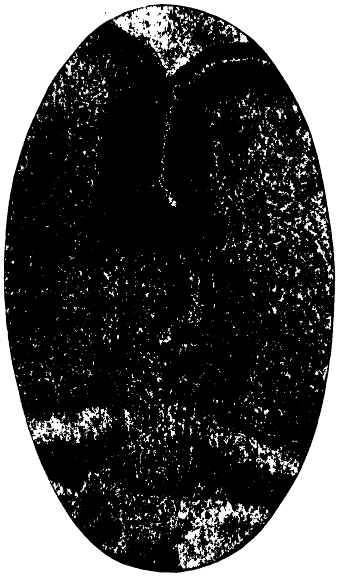

FIG. 11.

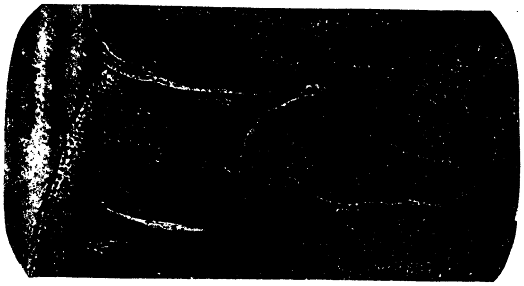

FIG. 12.

PLATE III.

FIG. 13.

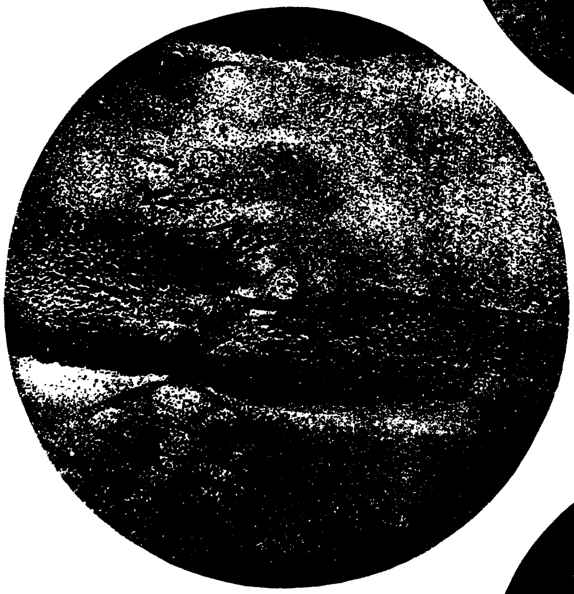

FIG. 14.

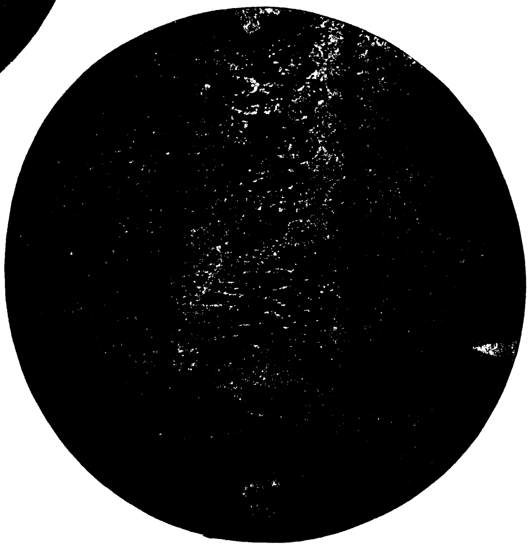

FIG. 15.

PLATE IV.

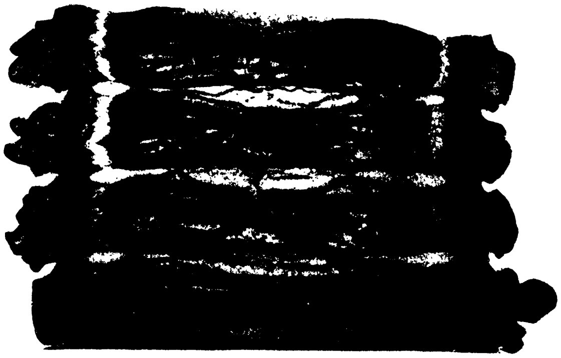

FIG. 16.

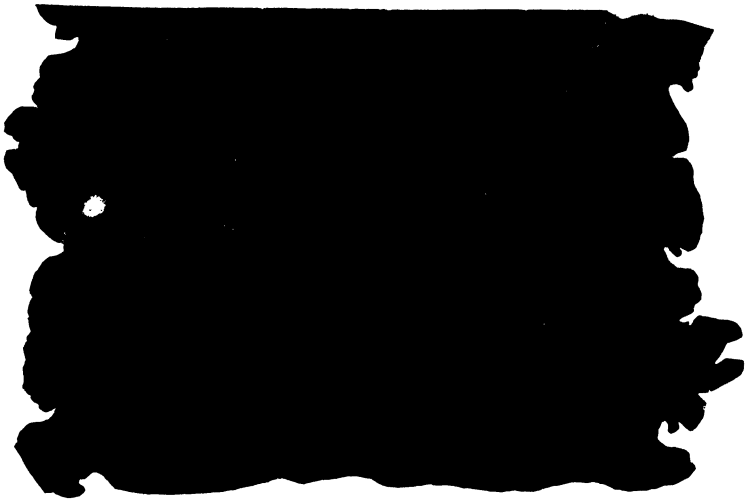

FIG. 17.

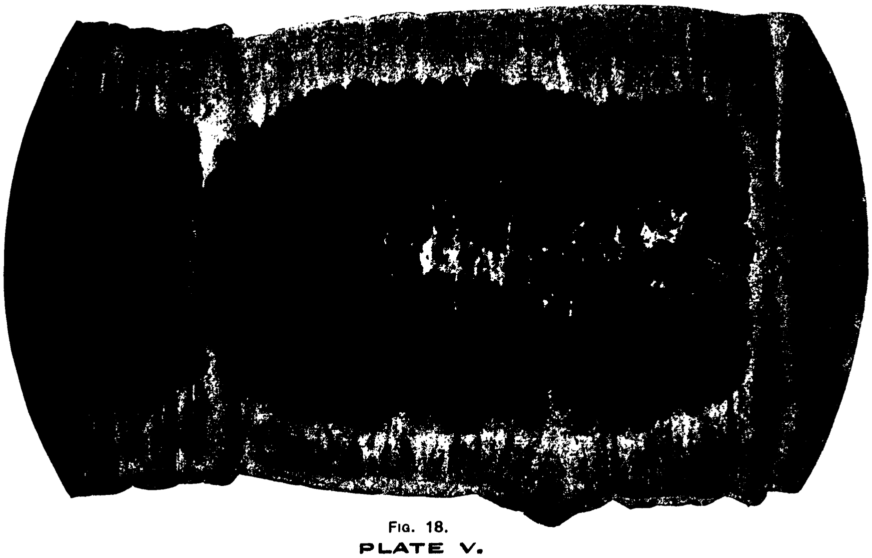

FIG. 18.

PLATE V.

NOTES ON CHRONIC ULCERS OCCURRING IN THE PHILIPPINES.

By George Cheyne Shattuck.

(From the Biological Laboratory, Bureau of Science, Manila, P. I.)

On the occasion of a recent visit to Manila opportunity was afforded me to work in the biological laboratory of the Bureau of Science and I became interested in attempting to determine whether or not Oriental sore occurs in Manila and in investigating the etiology of tropical ulcerations in general, as the question of etiology at present is in a state of much confusion.

This paper, embodying the results of my investigations, is based on clinical notes and microscopic findings in thirty-four cases of chronic ulcerative processes. With the exception of one Chinaman, all the patients were Filipinos. Fifteen of them lived in or near Manila,[1] and nineteen in the neighborhood of Catbalogan, on the Island of Samar. The Catbalogan cases were found among a number of natives suspected of leprosy who were being inspected by Dr. Heiser, Director of the Bureau of Health. They were held under observation for one week, and the ulcers examined repeatedly. Leprosy was not present in any of the patients of this series. In the Manila cases, smears were taken from the exudate on the surface of the ulcer and from the deeper tissues exposed by the curette. A piece of tissue[2] was removed and sectioned in four of these cases. In all the Catbalogan cases smears were taken from the exudate on the ulcer and from blood or serum expressed from a needle puncture close to the lesion. Giemsa's(9) new stain was used on the smears and a variety of stains were employed for the sections.

Table I.—*Catbalogan cases.*

[r.=right; l.=left.]

No.	Location of ulcers and scars.					Other lesions.
	Legs.	Feet.	Arms.	Hands and shoulders.	Other places.	
1	Scar, r	Scar, r				Hypertrophic periostitis, tibia, r.; contracture of toes, r.

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[1] Twelve cases are from Dr. Saleeby's clinic at St. Luke's Dispensary, two from the service of Dr. Dudley at St. Paul's Hospital and one from that of Dr. Edwin C. Shattuck at the hospital of Bilibid Prison.

[2] Tissue was first put in alcohol and subsequently treated with acetone and embedded in paraffin. Thickness of sections 4 μ.

TABLE I.—*Catbalogan cases*—Continued.

[r.=right; l.=left.]

No.	Location of ulcers and scars.					Other lesions.
	Legs.	Feet.	Arms.	Hands and shoulders.	Other places.	
2	Scars, r. and l.; ulcer, r.	Scar, l		Scars, shoulder; ulcer hand, r.		Amputation of second finger, r.
3	Scar, ankle	Ulcers, l			Soft palate and uvula gone.	
4	Ulcers, r. and l.	Ulcers, r. and l.				Two toes amputated, r.; contractures, knees, and foot, l.
5	Scars, r. and l.; ulcer, l.		Scars, wrists	Scars, shoulders and hands.		Finger amputated r.
[a]6						
7	Scars, r. and l.; ulcers, r. and l.		Scars, r. and l.		Scar, nose	
8	Scars, r. and l.; ulcers, ankles.		Scar, l			Two toes amputated, r.
9	Scars, r. and l.; ulcers, r. and l.	Scars, r. and l.	Scars, elbows			Contracture legs.
10			Scars, r. and l.; ulcers, elbow, l.			
11	Scars, ankle l.; ulcers, ankle, l.		Scar, l		Scars, forehead.	Bones of forearm half destroyed; l. epitrochlea.
12	Scars, r. and l.	Scars, r. and l.; ulcer, r.	Scars, elbow	Scars, shoulders.	do	Bone destruction, forehead.
13	Scars, r. and l.; ulcers, r.		Scars, r. and l.; ulcer, wrist, l.	do	do	Do.
14	Scars, r. and l.; ulcers r. and l.	Scars, r. and l.	Scars, elbow	do	do	Do.
15	Scars and ulcers, l.; scars knees.	Scars, r	Scars, l.; ulcers, l.	Scars, shoulder, l.	Scars, cheek	
16	Scars and ulcers, ankle r.; scars, l.					
17	Scars and ulcers, l.					Contracture, foot, l.
18	Scars and ulcer, r.					Hypertrophic periostitis, tibia l.; hand swollen and deformed.
19	do	Scars and ulcers, r.				Hypertrophic periostitis, tibia l.

[a] No. 6 was not tabulated.

TABLE II.—*Location and character of lesions in Manila cases.*

[r.=right; l.=left.]

No.	Legs.	Other places.	Miscellaneous.
[a]14319	Ulcer, ankle, r		
[b]2697		Ulcer, perineum, groin, penis.	General glandular enlargement.
[c]6536-3-P	Scars, r. and l.; ulcer, l		
[a]14612		Ulcer, buttock, l	
[a]14584	Scars, r. and l.; ulcer, r	Inguinal glands	
[b]1495	do	Scars, vulva	
[a]14125		Scars, foot and arm, r	
[a]11778	Scars, r. and l.; ulcer, l	Ulcer, palate	Hypertrophic periostitis, tibia, r.
[a]14413	Scars, r. and l.; ulcers, r. and l.		
[a]14518		Scars and ulcer, foot, l	
[a]13842	Scars, r. and l.; ulcers, r. and l.		
[a]12846		Ulcer, sole of foot	
[a]14560	Ulcer, ankle		
[a]13647	Ulcer, l		
[a]14581		Scars and ulcers, foot, l	

[a] St. Luke's Hospital number. [b] St. Paul's Hospital number. [c] Bilibid Hospital number.

Strong(1) described three very different types of ulcer found in Manila, exclusive of those due to tuberculosis, leprosy or syphilis. His first two cases can best be discussed in relation to the first and second cases described below. His third variety, observed in whites only, was not found in this series. The report of cases follows.

FIRST TYPE OF ULCERATION.

CASE I.—Native,[3] age 30, a laborer. Neither the patient nor his family have previously had any similar disease. Patient stated that four years ago, without assignable cause, a small boil appeared on his foot, that it burst spontaneously and did not heal. When he began coming to the dispensary, a month before I saw him, a diagnosis of phthisis was made. The patient denied that anything had ever been done except to apply dressings but when Dr. Saleeby went on service about that time he removed a stitch which was in the base of the ulcer. The patient was fairly well nourished, had no scars suggesting syphilis, and only the one external lesion. This was situated just below the external malleolus on the right foot where there was an ovoid swelling 2.5 × 2.5 × 0.75 centimeters in size, with a gash across it that went down nearly to the periosteum. The walls of the gash were somewhat ragged and the lower side was undermined. A little, yellowish slough was adherent to the base and sides. There was scarcely any discharge. The superficial skin over the swelling was thick and rough. The subcutaneous tissues were thickened. The swelling was firm. There was no tenderness, redness nor pigmentation. The lesion was curetted and a small piece excised for examination. The wound healed rapidly under simple dressings.

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[3] St. Luke's Dispensary, No. 14319. First seen March 30, 1907.

A smear from the scrapings showed many polymorphonuclear leucocytes, a few large and small basophiles and occasionally a plasma cell. A very few cocci were observed, but no other bacteria nor parasites. In addition, a few round bodies identified as blastomyces by their contour and budding were evident in the smear from the exudate. A section stained with hæmatoxylin and eosin showed all the layers of the skin and a little subcutaneous tissue. Ulceration, infiltration, and necrosis of the upper layers of the skin appeared at one edge of the section. There was an increase of dense, fibrous tissue in the deeper parts of the sections and strands of this tissue ran out to the edge of the ulceration. The tissue near the ulceration was œdematous. Coagulation-necrosis, deposit of fibrin and considerable infiltration with small round and plasma cells and a few polymorphonuclear leucocytes was present. Other sections were stained for tubercle bacilli, as well as by the methods of Wright, Giemsa, Gram-Weigert, and silver impregnation. A few Gram-staining cocci were seen near the edge of the ulcer, but no blastomyces.

This lesion corresponds pretty closely to Strong's description of his "ulceration of the first type." On the one hand, the scarcity of pus is common to both, and the mode of onset is much the same; on the other, Strong encountered an oval blastomyces in the tissues, whereas in this case a round one was found sparingly in the exudate. It must be borne in mind that in this case the condition had existed for four years when it came under treatment, and that probably the lesion had been thoroughly curetted when the stitch was put in before specimens were obtained. Supposing it to be of parasitic origin, the parasites may either have died or have been scraped out. The duration of the ulcer in this instance was four times as long as is usual with Oriental sore. The finding of blastomyces in the exudate, which might have been secondary invaders, is of no value, and yet the assumption of a blastomycotic origin would explain the chronicity and mildness of the lesion better than any other diagnosis I can make.

SECOND TYPE OF ULCERATION.

CASE II.—Native, age 21.[4] Patient says that five months ago he had swellings in both groins which disappeared under treatment by a Chinese physician. There was also a sore on his penis. Then a small boil appeared in the right groin, resulting in an ulcer which spread slowly in spite of treatment. Another ulcer developed later between scrotum and thigh. The patient was treated for a month in the out-patient clinic and was admitted to the wards a month ago because the ulcers were obstinate. At the time of entrance he had a large chancroid on the penis. The chancroid has improved, but the large ulcer has remained in spite of vigorous anti-syphilitic treatment. On the contrary, several small ulcers have appeared recently near the anus.

The patient was fairly well nourished. He had no skin lesions other than those already mentioned. There was slight, general, glandular enlargement. The throat was negative and there was no periostitis. Extending from the base of the scrotum to the inner aspect of the right thigh was a smooth, granulating area neither elevated nor depressed and measuring about 10.5 centimeters in greatest diameter. Its outline was made up of curves like the arcs of intersecting circles. The margin was clearly defined, a little raised and slightly undermined. The surface of the granulations was level; they were boggy and dark bluish-red in color. No slough was visible. Several round, ulcerated papules appeared near the anus. Their interior resembled the surface of the large ulcer. There was an ulcer of moderate size in the groin which appeared to be improving. Dr. Dudley curetted the large ulcer and those near the anus. The granulations were so soft that they could almost have been wiped away. The fascia beneath appeared healthy. A week later the ulcer was covered with healthy granulation tissue, but at the end of the second week the surface was uneven and covered with small patches of grayish slough. The granulations at the edge were slightly exuberant, and those in the other portions appeared as if gnawed away. The color of the granulation was light red. The ulcer of the groin presented the same appearence. There had not been much change in the size of the ulcers.

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[4] St. Paul's No. 2697. First seen April 24, 1907.

Smears from the ulcerated surface showed ordinary pus and a few diplococci, but no other bacteria. Smears from the depths showed neither bacteria nor parasites. After curetting, agar cultures were made from the base in the ordinary way and under anaërobic conditions. They remained sterile. A culture on blood serum showed one colony of *staphylococcus* and several colonies of a white mould. A section stained with hæmatoxylin and eosin showed a rounded edge covered with epithelium and undermined by ulceration. The papillary layer of the epithelium was hypertrophied. The fibrous tissue of the corium was much increased and infiltrated with a few small round cells. There was a fibrinous exudate on the surface of the ulceration, having polymorphonuclear leucocytes and plasma cells in its meshes. Other sections were stained with eosin and methylene blue, by silver impregnation, by Gram's method, and for tubercle bacilli, but neither parasites nor bacteria were found in the tissues.

Like Dr. Strong's second case this ulcer began without previous injury, spread slowly in spite of treatment, and did not improve until after curetting. It differed clinically from Strong's case in having a smooth base and no slough, although the lesions may have been modified by the two months' treatment. The discoloration and bogginess of the surface recalls Scheube's (4) description of phagedenic ulcers. Against the supposition that this ulcer was due to the same organism as the chancroid, we have the fact that the lesions reacted differently to treatment, one healing and the other not. Syphilis is ruled out by the failure to respond to energetic treatment with iodide and mercurials. The appearance and behavior of the ulcer was not as it would have been if produced by tuberculosis. When first seen it differed from the description of ulcerative venereal granuloma (Menso and Manson) (2 and 3) in having a dull, bluish appearance, a smooth surface, a comparatively rapid development, and the absence of a tendency to heal in the center. Dr. Dudley is sure that until he curetted the ulcer it had neither been trimmed nor scraped nor cauterized, but only dressed. Its appearance was not that of ulcerative venereal granuloma. In favor of phagedena are the obstinacy of the ulcer, the discoloration of the granulation tissue, and the fact that common causes can be excluded.

THIRD TYPE OF ULCERATION.

Case III.—Native,[5] age 37. The patient states that he had smallpox long ago, but no other illness or skin affection until two years previously when he received a wound from a board on the left leg. A large ulcer developed from the wound and at about the same time others appeared spontaneously on the other leg. These healed, but the primary ulcer did not. The patient was unusually well developed and well nourished. There were no lesions in the throat, no glandular enlargement and no anæsthetic areas nor infiltrated nodules.

On the anterior surface of the left leg, midway between knee and ankle, was a smooth scar about 15 centimeters long and 0.5 centimeter wide. The skin over the scar was pale, and the hair along its margin was white. On the external aspect of the leg, surrounded by pigmented scar tissue, was a shallow, granulating area. The edges were neither elevated nor undermined. The surface was clean. There was a small ulcer on the front of the leg, covered with a viscid, purulent exudate and a brownish crust. Grouped around the right ankle were many pigmented scars. The man was put on small doses of potassium iodide the day before I saw him and antiseptic dressings were used. When he was again seen six weeks later, the large ulcer had a border of new skin around the edge, and the granulations in the center appeared healthy. Two smears from the exudate of the small ulcer were examined. None were taken from the large ulcer because it had been cleaned. They showed polymorphonuclear leucocytes in abundance, tissue fragments and very numerous cocci and bacilli, some lying intra- and some extra-cellular.

The history and course of this case is typical of many others in which chronic ulcers developing after trauma have been followed by ulcerations in other parts of the body, particularly on the leg. It may be seen that lesions occurring in this manner are not all alike by referring to the second case described under the fourth type of ulceration. Some cases gave little indication of syphilis, but others had lesions suggesting this disease strongly. The second case[6] (Case V, p. 557) of the fourth type of ulceration is such a one.

Mense (3) states from personal observation that leg ulcers from trauma are very common in Africa among the negroes. He says that they heal without antisyphilitic treatment, are often large, but *generally single*. In our case the balance seems to swing toward syphilis as the most probabie diagnosis, but there is much room for doubt. The bacteria observed were probably nothing more than secondary invaders. In favor of syphilis are the multiplicity of the lesions and their close resemblance to other lesions seen in undoubted syphilitics. Other characteristics are ambiguous.

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[5] 6536–3–P, Bilibid Prison. First seen March 22, 1907.

[6] St. Luke's No. 14584.

FOURTH TYPE OF ULCERATION.

CASE IV.—Native,[7] coachman, age 33. Patient denied having had any skin lesion until three years ago when he was scratched by harness. An ulcer developed at the site of the scratch and gradually increased in size. The patient appeared healthy and showed none of the ordinary signs of syphilis. The lesion was roughly circular, 17 to 20 centimeters in diameter and occupied most of the posterior surface of the right buttock. It extended a short distance across the median line to the left buttock. The skin around the lesion appeared normal. The margin of the lesion was elevated and composed of a dense, inelastic tissue covered with pink, scaling epidermis, which was adherent and immovable. The same sort of tissue covered the greater part of the lesion. The ulcers near the margin were small, deep, pit-like and of uniform size. Their edges were firm, inverted and slightly undermined. They contained sero-purulent exudate and there was a yellowish slough at the base of each. In the central part of the lesion the ulcers were deeper, larger and serpiginous as if formed by confluence of smaller ones.

A smear showed polymorphonuclear leucocytes in fair numbers, a few large basophilic cells, and an occasional small lymphocyte and eosinophile. A few diplococci were observed but no other organisms. A section taken from the edge of an ulcer, stained with hæmatoxylin and eosin, showed thickening of the Malpighian and papillary layers of the epidermis and vacuolation of some of the cells of the upper *stratum lucidum*. The normal pigment in one place was wanting. The reticular stratum appeared œdematous and showed a marked increase of connective tissue cells. The lymph spaces were wide and contained polymorphonuclear leucocytes and plasma cells. There was no small round-cell infiltration about the veins and no endarteritis. The epidermis at one end of the section was undermined by ulceration. At this point traces only of the structure of the corium remained. There was much fibrinous exudate. Other sections stained by the Gram-Weigert method and for tubercle bacilli show many diplococci and a few bacilli in the horny layer but none in the deeper tissues. No tubercle bacilli were found and a section stained with silver showed no spirochætæ.

The patient was put on small doses of potassium iodide and the lesion was cleaned and covered with a wet bichloride dressing. It appeared much better after two days and at the end of six weeks the remnants of ulceration were covered with a few small scabs. The skin over the site of the lesion was still pink, but the subcutaneous induration had disappeared and the skin was soft.

CASE V.—Native,[8] age 55. History unreliable. The patient states that he was kicked by a horse seven months ago on the left leg, and that a chronic ulcer developed from the wound. The right leg became ulcerated at about the same time. The patient was poorly nourished. There were no scars in the throat nor any typical mucous patches. The inguinal glands were somewhat enlarged and hard, but the other glands were not. On the front of the left lower leg was a large, serpiginous, pocketed ulcer. The pockets contained moist, grayish slough and necrotic granulation tissue. The margin was indurated and bands of firm, inelastic tissue covered with adherent skin intersected the ulcer. The left leg presented a few pigmented scars around the ankle. The ulcer was cleaned and dressed with an antiseptic. The ulcer when seen again two days later appeared very much better. A piece of tissue was removed from one of the knobs in the base of the ulcer. The patient stopped coming and has not been seen since.

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[7] St. Luke's No. 14612. First seen March 22, 1907.

[8] St. Luke's No. 14584. First seen March 20, 1907.

Smears from scrapings showed blood, with a few polymorphonuclear leucocytes and many cocci and thick bacilli, the latter intra- and extra-cellular. One slide also contained a few blastomyces. A section stained with hæmatoxylin and eosin showed hypertrophy of the middle and papillary layers of the epidermis. There were a few polymorphonuclear leucocytes and plasma cells in the corium, but no lymphocytes around the vessels and no endarteritis. No necrotic nor ulcerated areas were seen. The silver stain and that for tubercle bacilli gave negative results. The Gram-Weigert stain showed many diplococci and bacilli in the horny layer (the former were positive and the latter negative to Gram).

Case VI.—A young native woman.[9] The patient was poor and emaciated, the throat negative and the glands not enlarged. The lesions were confined to the legs and feet, which presented an appearance suggesting elephantiasis. The knees could not be completely straightened. The left foot was firmly held in the position of equinus by contracted, atrophied muscles. The right foot and ankle were much enlarged and held immovable by the dense, wooden character of the swelling. The skin was pink, unyielding and immovable with a macerated, scaling surface which merged into large ulcers encircling both ankles. There were sloughing, ragged ulcers of varying size and depth on the feet. The great and second toes of the right foot were absent, but the bone was not exposed. On the legs there were many small, round, punched-out ulcers of equal size, having firm, inverted edges and a yellowish slough at the base from which exuded a sero-purulent, sticky fluid. The legs were bathed in this foul-smelling exudate. They were not anæsthetic. This patient was under observation for a week. The lesions improved under simple washing.

Smears from the exudate taken on two occasions showed pus and enormous numbers of bacteria of many kinds. Spirochætæ were present in three out of four specimens and were very abundant in two of these. The fourth specimen contained blood with a little pus and only a few bacteria. Many of the spirochætæ could fairly be classed as *Sp. refringens* Schaudinn, but the characteristics of the majority were midway between these of typical *refringens* and typical *Treponema pallidum* Schaudinn. No typical examples of *pallidum* were seen.

No. 17 (see Table I) is an excellent example of the same sort of hypertrophy and ulceration. The toes on the affected limb were drawn up by contractures.

These four cases had two important common characters: First, a similar hypertrophic process affecting the skin in the same way; second, punched-out ulcers of the same type. These fundamental resemblances were noticeable at a glance. Stelwagon (6) in his description of ulcerating gummata says that an "elephantoid" condition with punched-out ulcers is common, that it has no clear limits and that in a few months it takes on a violaceous hue, softens, and breaks down or is absorbed. The indications are that the hypertrophic condition in the first and third cases has lasted much longer than the limit which this auther has given. The process in the first case was pretty definitely circumscribed, but its limits in the third case could not be determined. Chronic inflammation is shown in the sections. They are not characteristic of syphilis nor of tuberculosis. The bacteria and spirochætæ found in the smears probably were all secondary invaders, for the most part saprophytic. Their absence in sections points to this conclusion. If we concede that the four cases have the same etiology, we may say that they are due to a chronic, but curable, nontuberculous disease causing hypertrophy and ulceration, and that the disease is probably infectious. The diagnosis of syphilis is almost forced upon us. Blastomycotic infection and tertiary lesions of yaws are far less probable than syphilis. Elephantiasis does not require serious consideration as a possible diagnosis. Whether we do or do not concede that these cases have the same etiology, it must be recognized that they have many differences. The contracture and loss of toes in the third case and contracture in the fourth are not easily explained by assuming that syphilis alone is present. They may have resulted from long antecedent phagedenic ulcers which preceded the hypertrophic process. The present condition of the skin renders it difficult to judge of this by observation. Nerve leprosy seems less probable as an explanation in view of the careful consideration it was given and the negative verdict. Congenital defects and trauma are possible, but not probable, etiologic factors.

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[9] No. 4 of Cathalogan series. First seen April 10, 1907.

Table I of the Catbalogan cases shows (1) a strikingly large number of lesions on the legs, (2) multiplicity of lesions, (3) a marked resemblance in the distribution of lesions, and (4) a considerable number of deformities. Clinically, the resemblance between individual lesions in different cases and between individual cases themselves is very striking. This points to a common etiology.

Leprosy, tuberculosis, and syphilis require careful consideration in the diagnosis of this group. Against leprosy we have the facts that there were no signs such as loss of eyebrows, there were no nodules, spots, nor anæsthesia, and also six or more smears taken from inside the nose, the lobes of the ears and from the lesions in every case were negative. Were the bone lesions tuberculous, sinuses or typical tubercular lesions of the skin would have been present in some of the cases. Nothing of this sort was found and no tubercle bacilli were seen in the smears made for leprosy.

The following lesions: Destruction of soft palate, dactylitis, destruction of the phalanges, depressions in the bone of the forehead, and "elephantoid" swellings of the legs, with gumma-like ulcerations indicate the presence of syphilis. The microscopic evidence is negative. Syphilis, then, is a probable diagnosis, but syphilis, uncomplicated, rarely produces ulcerations deep enough to cripple limbs or amputate toes. According to Scheube, tropical ulcerating phagedena does this very thing. It commonly invades unprotected lesions in the Tropics. Therefore, the diagnosis of syphilis complicated by phagedena might be made.

TABLE III.—*Tabular summary of all cases.*

Location and character of lesions.	Number of each.	Percentage of each.
Ulcers or scars of lower extremity	32	94
Ulcers or scars of upper extremity	13	35
Ulcers or scars in other locations	14	41
General glandular enlargement	1	3
Hypertrophic bone lesions	4	12
Destructive bone lesions	4	12
Amputations	4	12
Contractures	5	15

Table II of the Manila cases shows the preponderance of lesions on the legs, as is the case in Table I, and also demonstrates that the number and variety of lesions in the Manila cases was much less than in those from Catbalogan. Their essential characters are the same.

The spirochætæ which were seen present some interesting features. The organisms were found in five of the thirty-four cases (about 15 per cent). There were several varieties which may be divided into three classes.

TABLE IV.—*Occurrence of spirochætæ.*

Case No.	Class A.	Class B.	Class C.
4	Numerous	Abundant	Rare.
8	Few	Numerous	
14	do	Few	
14560	do	Numerous	
12846	Numerous	Abundant	

Characteristics of Class A: 1. Outline wavy, rather than spiral. 2. Curves very large and sweeping. 3. Curves few, rarely more than six. 4. Body wide and short. 5. Body as a whole nearly straight. 6. Ends gradually tapering to a point. 7. Stains dark blue with Giemsa. 8. Stains heavily.

Characteristics of Class B: 1. Outline wavy rather than spiral. 2. Average length of curve is moderate. 3. Curves shallow. 4. Number of curves rarely more than ten. 5. Curves often of different sizes in same individual. 6. Body often bent, curved or looped. 7. Body of medium thickness and medium length. 8. Both ends tapered as a rule; sometimes one end is truncated. 9. Stains blue or purple with Giemsa.

Characteristics of Class C: 1. Outline wavy or spiral. 2. Curves very short. 3. Curves shallow. 4. Number of curves rarely more than fourteen. 5. Curves of nearly uniform size. 6. Body as a whole nearly straight. 7. Body very thin and long. 8. Ends tapered. 9. Stains light blue with Giemsa; sometimes purple. 10. Stains faintly.

Evidence of transverse division was observed in all three classes. It was indicated by a pale area between two curves or at the top of a curve near the middle of the organism (Pl. I, fig. 1, *a*), or at two points in the same organism, dividing it into thirds. The pale area appeared to be narrower than the rest of the body. This is particularly well shown in examples of Class A, when the division, if such it be, is nearly complete. It might be argued that two individuals happening to lie end to end would produce a false impression of transverse division, but against this assumption are the facts that many instances can be found in a single specimen and that the line of the curve is unbroken. These appearances occur, but they are by no means as distinct in Classes B and C as in Class A; similar ones have been described by Goldhorn (8) and Fox (5) for *Treponema pallidum,* and by Novy and Knapp (7) for *Spirochæta obermeieri.* Classes B and C also at times suggest longitudinal division or agglutination, or both. A very few examples were observed in which one end of the organism was distinctly forked. Two organisms, intertwined, were not uncommonly seen, but in many of these instances four separate ends could be distinguished, so that the arrangement might have come about either by longitudinal division or by agglutination. One large bundle of organisms of Class C was observed, the individuals being arranged nearly parallel to each other. One *spirochæta* projecting from the side of the bundle showed a forked extremity, with the junction of the ends entirely clear of the bundle where it could plainly be seen. As the number of specimens which I have examined is a small one, these data on the question of multiplication of *spirochætæ* are not considered to be sufficient to prove the occurrence of either form of division.

I could not establish the causal relationship between the *spirochætæ* and the ulcers.

SUMMARY.

Four different types of ulceration were studied. The first and second types were not definitely diagnosed. They were probably infections *sui generis.* In the third type the weight of evidence is slightly in favor of syphilis. Many examples of this were seen. In the fourth type the probability of syphilis is strong. The remaining cases of the series seem to be variations of types three and four, which might all be placed in one group. Together they would comprise 94 per cent of the series (thirty-two cases). The diagnoses were all made clinically, because the microscopical findings were negative or ambiguous.

The cellular content of the exudates was nearly the same in all the cases. The bacteria were as a rule also identical.

Blastomyces were found in the exudate from two cases, but were not proved to be of etiological importance.

Spirochæta refringens and two other varieties of *spirochætæ* were observed, but they were not believed to bear a causal relationship to the ulcers. Evidence of division was seen among the spirochætæ.

Microscopical findings in brief.

	Number of cases.	Percentage.
Pus and pyogenic bacteria	34	100
Spirochætæ	5	15
Blastomyces	2	6

CONCLUSION.

Oriental sore as described in the text-books, Madura foot, and the typical phagedenic ulcer of Manson and Scheube were looked for in vain. No varicose ulcers were seen. The proportion of ulcers due to typical phagedena, blastomyces, and infections *sui generis* is small. A very large proportion of the chronic ulcers are syphilitic. Owing to neglect, the lesions are unusual in degree if not in kind, and they become very destructive.

The view that *Spirochæta refringens* is a bacterium is supported by strong evidence of transverse division.

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ILLUSTRATIONS.

(Photomicrographs by Mr. Charles Martin, Bureau of Science, Manila, P. I.)

Plate I.

- Fig. 1. *Spirochæta refringens* (Class A) giving appearance of transverse divison nearly complete; also an unidentified spirochæta of Class B. (1,000 diam.)
- 2. *Spirochæta refringens* (Class A) showing signs of transverse division. (1,000 diam.)
- 3. Spirochæta of Class B, one of them showing a forked end suggesting longitudinal division. (1,000 diam.)
- 4. Twisted examples of Class B. (1,000 diam.)
- 5. Spirochæta of Class C, which approaches *Treponema pallidum*, side by side witn *Spirochæta refringens*. (1,000 diam.)
- 6. Spirochæta of Class C, undergoing division. Separation incomplete. (1,200 diam.)

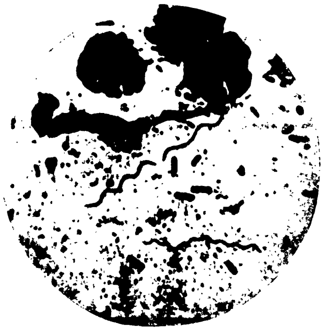

Fig. 1.

Fig. 2.

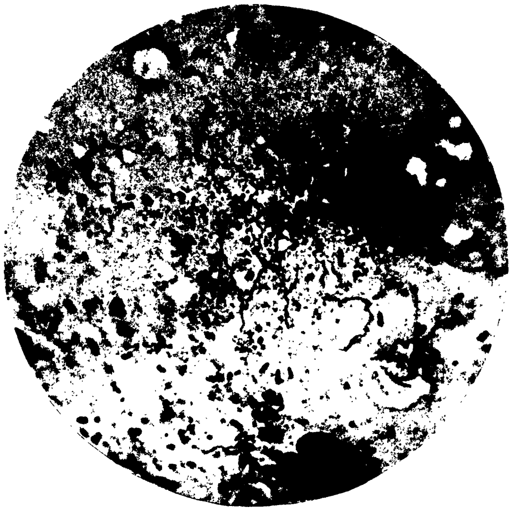

Fig. 3.

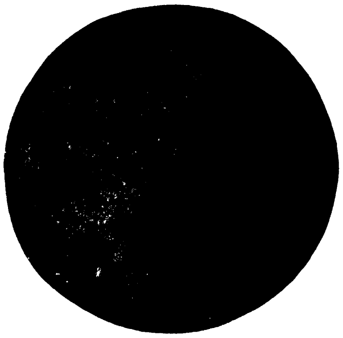

Fig. 4.

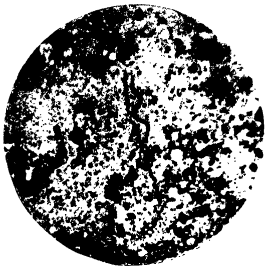

Fig. 5.

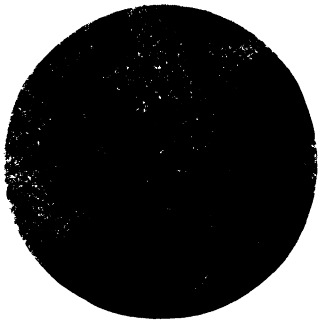

Fig. 6.

PLATE I.

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